INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

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The Botanical Review

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THE BOTANICAL REVIEW

VOL. XII JANUARY, 1946 No. 1

DETACHED LEAF CULTURE

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Detached leaf culture is here defined as the maintaining of leaves in a living condition for various periods after detachment from the plant of which they were once a part. Culture is here used to indicate life or existence as used by Vickery *et al.* (279) and Mason and Phillis (170) and not to indicate indefinitely continued growth as used by Carrel (41) and White (295), since leaves have apparently never been cultured in the meaning of the word used by Carrel and White. In detached leaf culture, investigators may be primarily concerned with the development and function of the leaf itself, with some parasite on the leaf, or even with some hyperparasite on a parasite on a leaf. The use of detached shoots is arbitrarily included in this review, but embryo culture is excluded.

No claim to completeness of coverage is made, and the writer would appreciate having called to his attention any important publications in the field of detached leaf culture which are omitted from the literature cited for this review.

According to Mathuse (171), Madriola in 1652 reported the formation of roots and shoots by detached leaves. In 1754 Bonnet (26) published a book on the function of leaves, in which he reported in some detail many tests with detached leaves. While his interpretations of the functions of leaves are not acceptable to resent investigators, his extensive data of the length of life of tached leaves held under various conditions of culture are still lid. Beginning about 1850 detached leaves came to be exten- ely used in studies of water absorption, transpiration, respiration d photosynthesis. The discovery by Boehm (24) in 1883 that tached leaves in the dark could form starch from sugar solution pplied to their petioles or to leaves floating on the solution was a great impetus to the use of detached leaves in studies of organic nutrition. Farlow in 1885 (84) was one of the first if not the first to successfully use detached leaves for inoculation tests with parasitic fungi, and Mains in 1917 (159) was the first to demonstrate clearly by means of detached leaves the important relation of carhobydrate nutrition of the host to the development of a plant pathogen (corn rust). Detached leaf culture is now a convenient technique for many plant physiological, phytopathological and entomological studies.

PHYSIOLOGY OF DETACHED LEAVES AND EFFECT OF DETACHMENT ON NORMAL LIFE PROCESSES

Length of life and process of death. The early death of detached leaves is the greatest obstacle to their use. When leaves are detached from a plant they usually die within 24 hours if deprived of moisture, or within 24 days if adequate moisture is supplied, but some detached leaves have been kept for surprisingly long periods. Under the best environmental conditions used, the maximum length of life of various detached leaves has been reported as follows: potato—five months (138), mint—several months (127), mulberry—almost six months (26), ivy—six years (173), cherry laurel—50 days (17), *Evonymus japonicus*—18 months (171), corn—14 days (159), *Solidago rugoso*—four months (48), apple—nearly five months (91), tobacco—12 days (279), red clover—17 weeks (306), *Rudbeckia*—14 days (129), lettuce—two weeks (193), bean—130 days (78). The best of the scanty information on the comparative length of life of detached leaves of different species is given by Bonnet (26) and Clinton and McCormick (48).

The periods of viability are mostly given by investigators who apparently wish to emphasize the long life of detached leaves; the minimum and average periods, unfortunately, are usually not stated. It is noteworthy that all periods of long life are with dicotyledons, and it is known that detached leaves of monocotyledons are generally shorter lived.

While detached leaves usually die sooner than attached leaves they can sometimes be made to live much longer than normal leaves (127, 171, 172, 247). Waters (286) states that no leaf was tried which could not be kept five or six weeks by his method.

Detached leaves supplied with water decline in vigor over several days and finally die, but, with few exceptions (11, 100, 279, 280, 324, 325), the process has not been described. Leaves with their petioles in water and their blades exposed to an unsaturated environment usually first show decline as a wilting of the blade, which is apparently associated with an interference with the translocation of water; this may be caused by plugging of vessels with micro-organisms or by host secretions. Leaves with their blades in a moist atmosphere and not attacked by micro-organisms usually show decline as a chlorosis starting with leaf margins, leaf tips or veins (100, 280) followed by a browning of the tissues (280), though the browning may occur without prior chlorosis. With cherry laurel leaves supplied with water in the dark, Audus (11) found the disappearance of chlorophyll, associated with an increasing rate of respiration, to begin in about 15 days and to be 75% complete in 35 days. Fungi began developing on the leaves in about 25 days. Hartt (100) found that detached sugar-cane leaves with their bases in sugar solution in the dark became chlorotic from the tip down starting with the third day, dried from the tip down starting with the sixth day, and by the 14th day were almost completely yellow with about half the leaf dried. Beginning of chlorosis may be associated with exhaustion of available carbohydrates (324) and with protein hydrolysis (177, 183, 325), and death (browning) may be associated with an accumulation of ammonia (280, 325). Frequently the death of the leaves is caused by their invasion by fungi and bacteria (48). Vickery *et al.* (279) report that destruction of chlorophyll was accompanied by a sudden loss of ether-soluble material and that completion of yellowing was associated with a reduction in protein hydrolysis. Exudation of water and filling of the intercellular spaces with water were associated with the death of barley leaves (324).

Growth and cell division. Leaves may increase in area and thickness after detachment (125, 146, 171, 220, 244, 247, 251, 290), and this growth is due principally to an increase in the size of epidermal, palisade and spongy parenchyma cells (146, 171, 247) with no increase in the number of these cells. The increase in area may be as much as five times in three to six weeks (244), and four-fold increases in length have been reported (125, 290). Schwarz (247) found greatest increase in area from rooted detached leaves. In cases where cell size was measured (146, 247), the length of the cells was usually not so much as doubled after detachment, and it would therefore be of interest to follow the changes in cell size in leaves which show such great increases in size, as indicated above. Riehm (220) found that detached beet leaves grew at the same rate as attached leaves for the first three days, but after that the growth of the detached leaves declined rapidly below that of attached leaves. With nine species of plants he found that the average total increase in size of leaves after detachment ranged from a maximum of 64% with *Anthriscus* to a minimum of 2% with cyclamen. The maximum increase for a single specimen was 90% for onion. In 0.1 per cent Knop's solution or in 0.1 per cent sucrose, leaves grew more rapidly than in distilled water. As the concentration of the medium increased, the rate of growth decreased, but even on 4% Knop's solution or 21% sucrose the leaves remained healthy and some grew. Injury to leaves decreased growth. Most rapid growth occurred at 27° C., but the more rapid the growth the more quickly it ceased, so that the total growth was about the same at high or low temperature. Total growth as well as rate of growth was greater in darkness than in light. Growth at 150 mg. mercury was double that at atmospheric pressure. Growth was greater when the leaf was under water, and Riehm believed that any treatment that favored water absorption favored growth. Detached leaves which had ceased growing were induced to resume growth by cutting off the roots which had formed, by injecting the leaf with water or with sucrose solution, and by treatment with mercuric chloride or reduced atmospheric pressure.

Weintraub (290) cultivated detached oat leaves in a nutrient solution containing sugar and inorganic salts in darkness, and the detached leaves grew in length at about the same rate as attached leaves.

Schimper (244) found that nitrogen and calcium in the culture medium favored growth of *Pelargonium* leaves and that no growth of detached *Chenopodium* leaves occurred in the absence of nitrogen.

Detachment itself acts as a stimulus to growth for some leaves which have ceased to grow in the attached position (146, 171, 247). It would seem likely that this detachment stimulus might be associated with the normal pre-detachment turgor deficit in leaves

(278). While uninjured epidermal and parenchyma cells of the leaf blade do not usually divide after the leaf is detached, the division of cells in uninjured vascular tissue has been reported (63, 171, 247). According to Schwarz (247), cell multiplication of the vascular tissue was associated with the growth of the lamina and with the formation of roots.

Injury to leaves may stimulate cell growth or cell division, or both. La Rue (135) found that cell growth, generally without cell division, could be induced in detached *Populus* leaves in a moist chamber as a result of contact with the medium, or from contact with healthy leaves, with crushed intumescences, with extracts from *Rhizopus,* with pure indol acetic acid and with other treatments. La Rue suggests that leaf outgrowths showing cell enlargement require auxin for their formation.

At the cut surface of the petiole of detached leaves, callus may be formed by cell division prior to or without root formation (117, 133, 136, 156, 161, 207, 247, 251, 304). Roots may arise by cell division in the callus tissue or above it (117, 121, 156, 161, 251). Shoots may form at wounded or unwounded parts of detached leaves (96, 117, 158, 220). The presence of a dormant bud on a detached leaf (268) makes shoot formation more likely, but shoots may arise adventitiously on detached leaves (87, 117, 158, 287). The extensive detailed literature concerning the formation of roots and shoots by detached leaves and shoots is considered beyond the scope of this review.

Cell division may occur as a wound response in the lamina of leaves (12, 17, 133, 136), though the intumescences studied by La Rue on poplar (135) usually showed increase in cell size without cell division. La Rue (136) found that leaves of pteridophytes did not form callus on wounds, leaves of monocotyledons usually did not form callus on wounds, and dicotyledons frequently formed callus, though most species did not. He found that plants with great regenerative capacity usually formed callus.

Lamprecht (133) found that cell division in leaf wounds was greatest closest to the vascular tissue, especially the phloem, and that wound-stimulating substances could diffuse from the phloem and stimulate cell division of other leaves, including closely related species, occasionally other genera, but not of other families. Lamprecht believed that the capacity for cell division was reduced as the size of the leaf pieces was reduced, and the smallest leaf pieces on which cell division occurred were 1.5 mm. square in the case of *Bryophyllum.* Priestley and Swingle (207) discuss the healing process at cut plant surfaces.

Isolated leaf cells may remain living up to four months and may increase in size (21, 52, 95), and Schmucker (245) reports division of an isolated mesophyll cell of *Bocconia* to have given about a dozen cells. This brief unconfirmed report of cell division is questioned by White (295) who reviews the numerous failures in the culture of individual cells and tissues of plants. The toxicity of tissue juices for cells of the tissue (204) is an interesting obstacle to the study of culture of cells of higher plants.

While growth and cell division of detached leaves are thoroughly established from the literature they have not been observed by most investigators. Vickery *et al.* (279) record that they observed no evidence of growth of tobacco leaves, and in the writer's tests where the leaves were usually held on 10% sucrose, no growth was noticed. The reasons for this apparent lack of growth in many cases is not clear, but are probably associated with species used and with cultural conditions.

Translocation. When leaves are detached from plants, translocation is reduced or almost eliminated, and this is responsible for important differences in the physiology of detached and attached leaves. In normal attached leaves there is a rather regular diurnal variation in water (62, 126), inorganic solutes (201), fresh weight (62), dry weight (51, 62, 226, 307), carbohydrates (51, 62, 169, 199, 226) and proteins (43), the highest values for water usually occurring at night and for the other materials during the day. Starch and other carbohydrates show the most striking and important diurnal variation. Sachs (226) showed that sunflower and beet leaves detached from the plant at 5 a.m. were starch-free, while similar leaves detached at 6 p.m. the previous evening and examined at 5 a.m. had an abundance of starch though less than similar leaves examined the previous evening. Sachs considered the slight decrease in starch in the detached leaves as due to respiration. With beet leaves he found that nocturnal translocation occurred within the detached leaf from the lamina to the veins, but when the lamina was freed from the veins there was only an insignificant nocturnal decrease in starch in the lamina.

Sachs discusses the relative value of morning and evening harvested foliage for fodder, tobacco, tea and silkworm food.

When detached leaves are floated on water or nutrient solution or placed with their petioles in solution, apparently only an insignificant amount of materials escapes from the cut portion of the leaf (50, 61, 226, 240). This elimination of translocation as a result of detachment is likely because of injury to and healing action of the phloem cells, which Curtis (50) has likened to the stopping of bleeding of animals due to coagulation of the blood at a wound.

Simon (251) considered that the growth of callus tissue at the cut end of leaf petioles was due to accumulation there of foods which could be translocated to that point but no farther.

Isolation of attached leaves by removing all but one of the leaves on a plant produces changes in attached leaves similar to the changes due to isolation by detachment, and these changes may be associated with translocation. Goos (92) studied the behavior of single leaves on plants after all other leaves on the same plants were removed or darkened. He found that the length of life, rate of growth, rate of assimilation and rate of translocation were greater for such single leaves than for control leaves on plants retaining their normal complement of functioning leaves.

Absorption of water. Detached leaves may absorb water through their injured or uninjured surfaces. Leaves in nature might be considered to be in a regular state of water deficit, for when they are immersed in water or placed with petiole in water and the remainder of the leaf in a moist chamber, they normally and promptly attain a greater weight than at the time of detachment (31, 45, 130, 157, 278, 279). Such leaves were generally collected during the day, and it is known that the water content of leaves during the day is less than at night (62, 126). The normally changing water content of leaves must be considered in determining water-absorbing power of leaves, for obviously less water is likely to be absorbed by a leaf which is already almost saturated with water. Bouissingault (29) observed that leaves collected in the early morning absorbed less water than leaves collected in the afternoon.

Absorption of water by leaves through cut petioles or stems is so frequently recorded and used in transpiration tests (172, 179) that the fact need not be further documented here. The progressively slower rate of absorption and transpiration by some leaves with their petioles in water as compared with similar attached leaves is discussed under transpiration.

In tests of water absorption by the lamina, absorption through the petiole was usually prevented by holding it out of the solution (23, 26, 97) or sealing it with some material impermeable to water (31, 40, 129, 157), and water absorption or loss was determined by the weight change observed when normal or water-deficient leaves were weighed before and after exposure to moisture, with precautions to remove any adhering but unabsorbed water before each weighing.

Absorption of water through the uninjured surface of attached or detached leaves has been thus observed (23,, 31, 40, 53, 54, 97, 104, 129, 157, 257, 293, 297, 329). Dandeno reviews much of the very early work on water absorption by leaves (53). It has not been clearly shown that this power of water absorption by leaves plays any important rôle in the water economy of normal plants, and Duchartre (68) and Ganong (89) failed to detect water absorption, but water absorption by uninjured surfaces is necessary or desirable in many detached leaf culture studies.

Absorption of water as water vapor has been reported (29, 40, 53, 157), but was not detected by Brierley (31). Burt (40) found that detached leaves or shoots lost water in a light moist chamber but gained water in a dark moist chamber, and this observation is closely associated with transpiration into a saturated atmosphere (55, 66). In cases where comparison was made of water absorption from free water and from water vapor, the rate was much slower for water vapor (29, 31).

Tests of water absorption by the lamina of leaves have been performed under a variety of conditions. Hales's classical demonstration of water absorption by leaves (97), since repeated by others, was to immerse in water one fork of a leafy forked branch, while the other fork was exposed to the atmosphere. The exposed leaves of this branch with its cut end out of water lived much longer than leaves of similar branches of which none of the leaves was in contact with water.

Henslow (104) detached leaves in the late afternoon, exposed them to drying conditions and allowed them to wilt for two hours, weighed them and exposed them under conditions of dew formation. At 7 a.m. the following day the free water was wiped off and the leaves were weighed. With 28 types or species, Henslow found that all regained their turgor, and the actual weight increases ranged from a minimum of 0.57% with *Berberis* to a maximum of 35% with grass.

Boehm (23) found that he was able to revive wilted bean leaves by immersing them in water when it was no longer possible to revive them by adding water to the roots of the supporting plant.

Ulchla (278) believed that water absorption by leaf tissues was injurious to the tissues, and that continuous growth of detached plant tissue was unlikely to be attained without further fundamental information on water relations. In harmony with this he observed that the injury to detached leaves decreased up to a certain point as the osmotic pressure of the substrate increased.

In contrast to the findings of Ulchla, Mason and Phillis (170) found that the longevity of cotton leaf discs was proportional to the amount of swelling which occurred when they were floated on a mineral nutrient solution. Leaf discs floated on a mineral nutrient solution increased their water content by as much as 170% in 13 days, while similar discs on pure water decreased in water content. They believed that salts in the nutrient solution increased the hydration capacities of the proteins.

Krause (129) found that the greater the water deficit in leaf tissues the greater the rate of water uptake. Also, thick, hard or waxy leaves absorbed water more slowly when detached than thin, delicate, non-waxy leaves. Generally the rate of water loss of detached leaves in the tested environments was much greater than the rate of water absorption in distilled water, but with *Phaseolus coccineus* he found that rate of water gain on immersion in water was about the same as the rate of water loss in wilting.

Prillieux (208) records a case of recovery from wilting of detached leaves in a moist chamber which was associated with a decrease, not an increase, of green weight.

Krause (129) observed that leaf hairs or cells at the base of leaf hairs showed more intensive staining with neutral red than other adjacent epidermal cells, and considered that these leaf hairs and adjacent cells might well be the point of entry of free water into the leaf when the leaf was floated on a solution. Marloth (165) also believed that leaf hairs could absorb moisture. Zamfirescu (329) believed that free water entered leaves along the veins and readily penetrated to the vessels. These observations of Krause, Marloth and Zamfirescu are thus closely related, since in many species leaf hairs occur most abundantly along the veins.

Nakajima (187) found that water freed of air by heating or vacuum treatment was more readily absorbed by detached leaves than was water containing air.

Leaves immersed in or floating on water may become water-soaked, though no extensive treatment of this phase of detached leaf culture has been found. Sen and Blackman (249) observed that water soaking of immersed leaves occurred in darkness but not in light and believed that this was explained by the use of oxygen in the respiration of the leaf, while the carbon dioxide and nitrogen in the leaf were dissolved in water. Water soaking from a water spray is greater in light than in darkness, however, because of the opening of the stomata (64).

MacDougal (151) observed that dead leaf parts also absorbed water readily from a moist atmosphere, and this should be considered in studies of water absorption by leaves.

Transpiration. Detachment of turgid leaves may cause a sudden temporary rise in transpiration lasting only a few minutes (65, 86, 107, 118, 123, 132, 198, 250, 263) followed by a slow decline. This surprising increase in transpiration was first suspected from porometer values (57), later confirmed, and interpreted as due to a sudden increase in the opening of the stomata due to a release of water tension on detaching the leaves (56, 132). Such increased opening of stomata was not confirmed by direct observations (148) or by indirect determinations (129), though some investigators have shown a close correlation between transpiration and stomatal opening of detached leaves (148, 263). The sudden temporary rise in transpiration after detachment of leaves or shoots is usually not detected by ordinary weighing measurements when intervals of ten minutes or more between weighings are allowed, but is demonstrated by frequent accurate weighings, as with the torsion balance (107, 112, 123, 198). In many though not all cases accurately studied by Pfleiderer (198), no increase in transpiration after detachment was observed, but only a gradual decrease, as is commonly believed to be typical (38). A temporary rise in transpiration was observed, however, in each of 17 tests with three species described by Firbas (86).

If shoots were cut under water no temporary increase in porometer values (132) and presumably of transpiration occurred.

After the temporary rise in transpiration, if one occurs, detached leaves not supplied with an external source of water transpire at a progressively decreasing rate (86, 107, 123, 132, 198, 250, 263), which rate has been investigated mathematically (250) and correlated with leaf temperatures (323).

The amount of water which leaves may lose before wilting occurs apparently varies greatly with environmental conditions and with species. Bouissingault (29) observed no loss of turgidity when grape or sycamore leaves containing 66% water lost 13% of their water, but the leaves wilted if they lost 16% of their water. Knight (126), on the other hand, records extreme flaccidity of detached leaves resulting from a loss of 1% of their water content. Leaves may even recover from wilting while continuing to lose water (208). Even when detached leaves or shoots are placed with their stems in water after the stems have been cut in air or under water, a decline in transpiration usually occurs (29, 37, 88, 281) and probably continues until the leaf dies, unless the leaf forms callus and roots, in which case an increase in transpiration is indicated by Winkler (300). This decreasing rate of water loss for detached leaves makes them frequently unsuitable for transpiration tests, though there are great differences in the way different species respond.

Bouissingault (29) and Ganong (89) found that when detached shoots were subjected to increased hydrostatic pressure they wilted more slowly than shoots under normal pressure, but the fact that they wilted when attached shoots remained turgid indicates that some factor other than decreased root pressure was responsible for the decline of detached shoots.

Burgerstein (37) temporarily revived wilted detached shoots with camphor.

M'Nab (157) found that transpiration of detached shoots in different gases decreased in the following order: oxygen, air, carbon dioxide, nitrogen.

Biale (16) and Montermoso and Davis (182) showed that a diurnal rhythm of transpiration was exhibited by detached shoots or leaves in a constant environment.

Although guttation is generally considered as due to root pressure (179), Vickery (280) records guttation on the lower surface of detached tobacco leaves presumably without roots.

Respiration. After detachment, leaves continue to respire at a rate similar to that of attached leaves, but with increasing time from detachment the respiration rate of detached leaves shows certain carefully described patterns, including a general decline in rate associated with exhaustion of carbohydrates (11, 28, 130, 194, 260, 324). Garreau (90) states that the respiration of detached leaves is similar to that of attached leaves, but in Garreau's data the comparable respiration data of detached and attached leaves are apparently not for the same plant species. In unpublished tests by the writer the rate of respiration of bean leaves, expressed as milligrams of carbon dioxide per hour per gram dry weight of leaves at the end of the test, was 1.19 ± 0.11 for detached healthy leaves, 1.52 ± 0.16 for attached healthy leaves, 1.76 ± 0.24 for detached leaves infected with *Erysiphe polygoni,* and 1.80 ± 0.23 for attached leaves infected with *E. polygoni.* These comparable values were secured from separate determinations within 15 hours of detachment of the respiration of nine single leaves of each type specified, and do not show clearly any difference between attached and detached leaves.

Decline in respiration after detachment is not constant, and temporary but consistent rises in rate in the course of the general decline in rate are usually observed (11, 130, 324). Audus (11) found the respiratory rate of cherry laurel leaves decreased from about 0.3 mg. per hour per gram of fresh weight at the beginning of the test to about 0.1 mg. at 240 hours after detachment, then rose to almost 0.3 mg. at 600 hours, then fell to 0.15 mg. at 740 hours, and rose again. The rise after 240 hours was associated with observed breakdown of chlorophyll, and the rise at 740 hours was associated with increased fungus growth on the chlorotic senescent leaves.

Yemm (324) reported that the respiration of detached barley leaves in the dark rose for approximately 12 hours after detachment, then fell until about 40 hours, rose or interrupted its rate of fall after 40 hours, and then fell rapidly from 140 to 240 hours as the leaves turned brown. None of the variations in carbon dioxide production could be correlated directly with concurrent changes in individual sugars, but in beans the carbon dioxide produced was accounted for by total carbohydrate loss for the first 30 hours. Later as carbohydrates became scarce the respiration rate decreased and the respiratory quotient decreased, presumably due to the utilization of protein in respiration. With barley less than 25% of the carbon dioxide produced from the 40th to the 140th hour could be accounted for by carbohydrate loss.

The greater the amount of carbohydrates available to detached leaves the greater their rate of respiration (130, 194, 289). Krotkov (130) found that the greater the initial sugar content of the leaves, as determined by cultural and seasonal conditions, the greater the initial rate of respiration. Palladin (194) found that at the end of the night period green leaves which have translocated their carbohydrates, respire as weakly as etiolated leaves. Weevers (289) with green leaves and Palladin (194) with green and chlorotic leaves found that respiration was greater for leaves on sugar solution than for leaves on water.

In the tests of Vickery *et al.* (279) the cellulose content of detached leaves remained unchanged up to 300 hours, and cellulose was therefore apparently not utilized in respiration.

Wounding (219) or mechanical flexure without wounding (11), either of which may easily occur in handling leaves for respiration tests, may increase the rate of respiration. Audus (11) found that bending detached cherry leaves in a standard manner increased their respiration by about 30% at the beginning of the test, but progressively less as the time from detachment increased.

Fungus infection generally and virus infection with less certainty, increases leaf respiration. This will be treated in more detail under culture of parasites. Iljin (115) considered that the increased respiration from fungus infection might be basically similar to the increase in respiration on wilting.

Laurie (139) believed there was a correlation between low respiration rate and long life in cut flowers. He found that hydrazene sulphate, phoroglucinol and resinol reduced respiration rate, and thought that proper addition of these to the water might prolong the life of flowers.

Only a relatively small part of the carbohydrates produced in leaves by photosynthesis is lost by respiration in a normal 24-hour day under ordinary growing conditions, and this appears equally true for attached or detached leaves. Ingen-Housz (116) was one of the first to use detached leaves and come to this conclusion. Sachs in 1884 (226) indicated that the dry weight lost by respiration in one day was about one twenty-fourth of the dry weight gained by photosynthesis for detached leaves. Blackman and Matthaei (18) showed that over a range from about 10° to 35° C. the rate of carbon dioxide absorption per hour in light was about ten times the rate of carbon dioxide formation in darkness for detached sunflower and cherry laurel leaves. That factors other than rate of respiration in relation to available carbohydrates are important in determining the length of life of detached leaves in the dark, is indicated by the variation in the length of life of detached leaves of different species, and by the relatively short though significant increase in the length of life of leaves which follows the addition of supplemental carbohydrates to the substrate.

Photosynthesis. Most of our knowledge of photosynthesis, including the dry weight and gas exchange phases, has come from studies of detached leaves or shoots (18, 32, 33, 34, 83, 106, 109, 113, 114, 116, 212, 216, 226, 238, 239, 240, 254, 255, 256, 258, 260). The literature is too extensive, therefore, to be reviewed here, since photosynthesis is only one of numerous phases of detached leaf culture. We are intimately concerned, however, with the comparative photosynthesis of detached and attached leaves, and the photosynthesis of detached leaves over long periods, though unfortunately these phases have not been extensively studied, nor are the available data entirely satisfactory.

Sachs (226) found by his half leaf template method that detached sunflower leaves in full sunlight increased in dry weight at the rate of 1.64 g. per hour per square meter. By the same technique he found that attached leaves increased in dry weight at the rate of only 0.91 g./hr./m.^{2} By adding an estimated translocation of 0.96 g./hr./m.^{2} out of the leaf during photosynthesis of the attached leaves, Sachs's value for attached leaves became similar to that for detached leaves. The rate of translocation during photosynthesis was based on uncorroborated assumptions, however, and what was otherwise a careful comparison of attached and detached leaves cannot be considered adequate. Even the half leaf template method of Sachs has been severely criticized (258, 273).

Brown and Morris (33) confirmed Sach's finding that the increase in dry weight of leaves in light was greater for detached than for attached leaves, but they found that starch did not accumulate as rapidly in detached as in attached nasturtium leaves, a result which is difficult to reconcile with the above and with the idea that starch is formed when soluble carbohydrates become abundant.

Brown and Escombe (32) found that detached sunflower and *Catalpa* leaves assimilated about 82% more carbon dioxide per unit area than similar attached leaves, and suggested that the increased photosynthesis was due to the greater opening of the stomata on detached leaves. This explanation of the discrepancy has apparently not been established. In simultaneous determinations of assimilation by carbon dioxide absorption and dry weight increase for the same detached leaves, Brown and Escombe found that the rate, as indicated by dry weight increase, was about three times that indicated by carbon dioxide intake. Saposchnikoff (238), however, found less dry weight than expected on the basis of the amount of carbon dioxide absorbed. By careful methods, Smith (254, 255) has recently demonstrated concurrence of carbohydrate formation and carbon dioxide utilization by detached sunflower leaves.

Elimination of translocation from the leaf to other plant parts in the case of detached leaves is responsible for at least some of the observed differences in assimilation of attached and detached leaves. Saposchnikoff (240) and Ewart (83) have shown that the rate of photosynthesis of detached leaves decreases as the carbohydrates of the leaf are increased and that in strong light the carbohydrates of detached leaves may reach as high as 35% of their dry weight, which is more than that attained by attached leaves, and that photosynthesis entirely ceases in leaves with such high accumulations of carbohydrates. Such carbohydrate-gorged leaves assimilated actively again after being held in the dark for three days (83).

Protein synthesis and degradation. Because translocation out of leaves is practically eliminated by detachment, detached leaves are ideally suited for studies of the nitrogen changes of leaves, but there is little comparative information of the nitrogen changes of detached and attached leaves. Although it was early believed that light was necessary for protein synthesis in leaves (183, 244), others (183, 184, 267, 327, 328) have shown that detached leaves supplied with sugar could synthesize proteins in the dark. Mothes (183, 184) found that young leaves synthesized protein from ammonium chloride and amides in the presence of adequate sugar, but as the leaves aged, as the sugar content decreased, or as the leaves were starved in the dark, protein synthesis ceased and protein decomposition began.

In detached leaves held in the dark and not supplied with supplemental carbohydrates, protein is gradually decomposed to amides and amines (45, 183, 260, 280, 325), to nitrates (154, 281) and to ammonia (183, 280, 281, 325), while the total nitrogen remains constant for long periods (61, 279). The liberation of ammonia is sometimes considered responsible for the death of senescent leaves (280, 325). Protein degradation is generally associated with carbohydrate exhaustion (61, 183, 266, 279, 281), but no sparing of protein by carbohydrates was detected by Yemm (325) or Chibnall and Sahai (45). The similarity of the nitrogen metabolism of detached leaves and of seedlings has been pointed out by Nightingale (191).

Takahashi (269) demonstrated that normal leaf protein but not tobacco mosaic protein was hydrolyzed to soluble nitrogen in detached leaves in the dark.

Chibnall (44) believed from his studies with detached leaves that asparagin was the chief form in which nitrogen in a form suitable for resynthesis of protein was translocated from one part of the plant to another, and Mothes (179) believed nitrogen was translocated in the form of amides.

Arisz and Oudman (8) found that absorption of asparagin by floating leaf discs of *Valisneria* occurred only in the presence of oxygen. The relative absorption was apparently greater from dilute than from concentrated solutions, for leaf pieces floated on 0.01% solution contained 0.36% asparagin after 72 hours, while leaf pieces floated on 0.66% asparagin contained 0.66% asparagin. Mothes (183) believed that the presence of asparagin increased the utilization of carbohydrates by detached leaves.

MECHANICS OF CULTURE

Different investigators have their favorite methods of culturing detached leaves, and these methods are in part determined by the peculiarities of the problem. All methods are extremely simple.

In studies of propagation, moist sand is commonly used as a substrate (127, 133, 265, 287). It has the advantages of being relatively free of decay-producing organisms, of holding sufficient moisture while allowing adequate aeration, and of excluding light from the root-forming cut end. The petioles are placed in the sand, and leaf blades are left above the sand surface. The bed of cuttings is usually covered so as to reduce but not to exclude light, and to maintain a high humidity. Root formation is sometimes inhibited by light (175, 247), but some leaf cuttings form roots readily in light (287). Techniques of propagation of leaf and other cuttings are given by Watson (287), and the general subject is beyond the scope of this review.

When regeneration is not desired, a common method for the culture of detached leaves has been in moist chambers, usually Petri dishes, on moist filter paper, with little consideration of light or carbohydrate supply (12, 19, 20, 227). Succulent leaves usually live only a few days under these conditions. Another simple method is to place the petioles of the leaves in water or nutrient solution, with the leaf blade exposed to ordinary air or the high humidity of a moist chamber (13, 14, 15, 18, 24, 42, 45). Leaves of Gramineae may be cultured with the base of the leaf immersed in the substrate in the bottom of a test tube and with most of the leaf exposed to the high humidity above the water (22, 100, 101, 277). Bonnet (26) laid the test leaves with most of the lower or upper leaf surface in contact with water but with the petiole and leaf margin projecting beyond the culture vessel and exposed to the atmosphere.

Floating of leaves directly on the surface of the aqeous substrate in a moist chamber is perhaps the most common method (8, 24, 48, 119, 286, 306). Hairy leaf surfaces are less likely to be wetted by the solution than non-hairy surfaces. If leaves are readily wetted by aqueous solutions the solution may creep over the leaf edge and on to the leaf surface which is facing up, and this seems to favor the growth of contaminants, to decrease the length of life of the leaves, and to reduce the growth of parasites, such as powdery mildews on the leaf surface. This tendency of the leaf surface to be undesirably wetted may be reduced by using a minimum amount of solution in the culture vessel, by using small culture vessels so that the culture solution is not unduly agitated in handling, by laying the leaf lamina on the surface of waxed paper which is in turn floating on the culture solution while the leaf petiole is immersed in the solution, by laying the leaves on paraffined metal screens (180, 286) through which the petiole projects to the culture solution below, by laying the leaves on absorbent cotton soaked with the nutrient solution (71), by holding the lamina away from the culture solution by glass rods or rubber bands (48, 286), by inclining the culture vessel so that most of the solution is confined to a small area, and perhaps by other methods.

Entire leaves or leaflets are usually used, but for certain purposes leaf pieces are more desirable. For quantitative comparisons of photosynthesis, Sachs (226) and Brown and Escombe (32) used half leaves, and this half leaf method has been critically studied by Thoday (273). In studies of water absorption Phillis and Mason (200, 170) used leaf discs, and these would seem ideal for many quantitative comparisons using detached leaves, especially when twin leaves or leaflets or their equivalent are not available. Dundas (78) placed pieces of several leaves in the same Petri dish chamber. In this way he was sometimes able to put representative leaf pieces from each plant of an entire progeny of a cross in a single dish, while similar sets of leaf pieces could be placed in other dishes, and all could be inoculated simultaneously with different strains of a single pathogen (*e.g.*, bean powdery mildew or rust) or with different pathogens. There is of course a limit to which leaves may be subdivided and will still function suitably in detached leaf cultures. Hartt (101) found that cutting cane leaves into pieces one inch long did not decrease sucrose synthesis, but grinding inhibited the conversion of dextrose to fructose and the formation of sucrose. Detached leaf pieces have been used also in other studies (3, 4, 8, 21, 52, 95, 133, 155, 215, 301, 302).

Onion leaves usually die in a few days after detachment, but Ewart (83) found that detached onion seed stalks with their bases in water would live for weeks, a fact used in breeding onions (122). Harris, Drake and Tate (99) sealed the cut ends of detached onion leaves with paraffin, but were able to keep such leaves in a satisfactory condition for only three to five days.

For very small leaves or leaflets such as those of clover, Syracuse watch glasses are ideal culture vessels and can be stacked in large numbers so that each dish forms the cover of the dish below it.

For larger leaves Petri dishes are quite suitable, and for still larger leaves special vessels are necessary.

Leaves or leaflets are usually cut from the plant with scissors and transferred with forceps; slide forceps or those with curved tips are more suitable than straight forceps.

Mechanics of culture primarily concerned with avoiding contamination are treated under contamination.

Adequate comparisons of the utility of different methods of culture have not been reported, but little difference in starch synthesis has been found, whether the leaves floated on the solution or were supported with only their petioles in the solution (42). Schimper (244) reported that leaves with their petioles in water remained fresh longer than leaves in a moist chamber, but Mothes (183) considered floating leaves more satisfactory.

CONDITIONS AFFECTING LIFE OF DETACHED LEAVES

Moisture. Detached leaves die from desiccation a few hours after detachment in a normal environment, as indicated by data given under "transpiration." In detached leaf cultures the leaves are ordinarily in a closed chamber in contact with water or some solution which maintains an almost saturated atmosphere. They will live for long periods, however, if only part of one surface is in contact with the liquid while the petiole and most of the leaf surface are exposed to the atmosphere, as in Bonnet's tests (26). Clinton and McCormick (48) considered that high humidity of the air in the chamber lengthened the life of the leaves, but they and other investigators seem not to have adequately established this point. Hitchcock and Zimmerman (105) found that detached carnations with their stems in water lived two to three times as long in an approximately saturated atmosphere as at 80% relative humidity. On the other hand, Ulchla (278) believed that water absorption by the leaf was injurious to leaf tissues. Lamprecht (133) found that high humidity favored rooting of leaf pieces, low humidity prevented cell division, and that an intermediate humidity favored callus formation.

Temperature. The optimum temperature for maintaining detached leaves in good condition is lower than for the growth of entire plants, but is not well defined. Clinton and McCormick (48) had difficulty maintaining detached leaves long enough in midsummer to secure infection with the rusts they were studying. Dundas (71) found that detached bean leaflets on 10% sucrose lived an average of 39 days at 8° C., 12 days at 25° C., and 7 days at 31° C. Hitchcock and Zimmerman (105) found that low temperatures increased the longevity of cut flowers of coriopsis, carnation and rose.

Boehm (24) found little difference in the amount of starch formed from sucrose at 10° to 20° C. In Chapman and Camp's studies (42), *Pelargonium* leaves showed increased starch formation from dextrose as the temperature was increased from 4° to 25° C.; the optimum was 25° to 38° C., the maximum about 50° C. Hartt (100) observed that absorption of dextrose or fructose by detached leaf blades of sugar cane remained about the same from 6° to 20° C., but increased from 20° to 40° C. The synthetic efficiency was 10% to 20% at 6° C., 72% to 83% at 20° C., 75% to 88% at 30° C., and 61% to 85% at 40° C. Leonard (143) found the highest synthetic efficiency at about 40° C., and sucrose was formed from dextrose at 50° C. Winkler (299) reported that the minimum temperature for conversion of sucrose to starch varied with the ecological type and season, and the maximum was about 45° C.

Roux (223) has described an unusual type of low temperature injury to detached leaves, manifested in loquat leaves as a browning and an increase in respiration.

The optimum temperature for the life of detached leaves may be a great deal less than that of any specific activity it is desired to measure, and it is likely that in many cases the higher the metabolic activity the shorter the life of the leaves. Considering both the life of the leaves and the leaf activity it is desired to measure, the optimum temperature for detached leaf cultures seems to be between 15° and 22° C. in most cases observed by the writer. It seems likely that the optimum temperature for detached leaves, like the maximum temperature for entire plants (137), might be lower in the dark than in light.

Light. Detached leaves exposed to light usually live longer than similar leaves in darkness, whether the leaves are supplied with water only (48, 78, 280) or with sugars (25, 71). Carbohydrates of leaves decreased more rapidly in the dark for leaves on water than for leaves on sugar solution (281). The two principal known effects of light on detached leaf cultures are its effect in increasing carbohydrates through photosynthesis and its effect in reducing the growth of contaminating fungi and bacteria (167), though other effects are quite likely. Lepeschkin (144) has shown that light increases the permeability of leaves to inorganic salts and to dyes, though this was not corroborated by Zycha (332), and Phillis and Mason (200) have shown that light too low for photosynthesis increases the starch content of leaf discs floated on sugar solution. Light is not necessary for the formation of either carbohydrates or proteins in detached leaves, however, and many tests are advantageously conducted in entire darkness. Riehm (220) found that detached as well as attached leaves grew more rapidly in darkness than in light.

Carbohydrate supply. If detached leaves are not cultured in light sufficient for adequate photosynthesis and if they are placed in contact with sugar solution rather than in contact with water, they usually accumulate more starch and sugar, probably show reduced exosmosis, remain living longer, have a higher rate of respiration, and maintain a more luxuriant growth of obligate parasites. This opportunity to manipulate the carbon nutrition of detached leaves independently of photosynthesis, first discovered by Boehm (24), was a notable contribution to plant physiology in itself and has been useful in many studies with detached leaf cultures.

Boehm (24) placed starch-free leaves in the dark with their petioles in sugar solution or with the leaves floating on the solution, and reported that by this treatment starch was formed in several species of plants, including four in which starch was not found in the leaves in nature. Leaves laid on 20% sugar solution formed more starch than leaves on 1% or 5% sugar solution, but there was little difference between 10% and 20% sugar. Leaves on 20% sucrose at 10° C. remained fresh for six weeks. Plants with roots but with cotyledons removed, lived longer in the dark or in a carbon dioxide-free atmosphere when the roots were immersed in a 0.25% to 5% sucrose solution than when the roots were immersed in water.

In most studies the path of absorption has not been considered, and cut leaves or leaf pieces have been floated on sugar solution or placed with their petioles in sugar solution. However, Schimper

(243) found that *Hydrocharis* leaves with their petioles held out of the sugar solution took up sugar solution through their laminas, and believed that the cells along the veins were especially active in absorption. Leonard (142), on the other hand, found no sugar absorption by beet leaves when only the laminas were in contact with the sugar solution and the petioles were in water. Waters (286) found that starch was deposited centrifugally from the bundles outward when cut pieces of leaves were floated on sucrose solution. The writer believes that sugar may be absorbed through uninjured leaf surfaces but that absorption occurs more rapidly through cut surfaces. Absorption of water and dyes by uninjured leaf hairs and by cells along the veins (129, 165, 329) would suggest a similar path of sugar absorption. The observation that clover leaflets increased in dry weight if the lower hairy surfaces were in contact with the sugar solution for 12 days, but decreased in dry weight if the upper relatively non-hairy surfaces were in contact with the solutions (307), would support the idea that leaf hairs facilitated sugar absorption in some way. Sugar may also be absorbed by roots and function to increase the growth of higher plants in light or in the dark (30, 128). If sugar is absorbed through the cut petiole, as in the tests of Vickery *et al.* (281), the sugar and water are absorbed in the same ratio as they exist in the nutrient solution, indicating that the sugar moves in with the transpiration stream, as would be expected.

Labile carbohydrates of leaves occur principally as starch, sucrose or dextrose, and absorbed or photosynthesized carbohydrates are usually converted to one or more of these. In dicotyledons, starch usually predominates, while in monocotyledons the reverse is true (24, 176, 197). According to Bokorny (25), starch is deposited when soluble carbohydrates reach a certain maximum which varies with species and other conditions. In the early work, formation of starch by destarched leaves was the criterion of sugar absorption, and it has been shown that green leaves may form starch from glycerine (1, 138, 176, 186, 237, 259), dextrose (1, 24, 42, 176, 186, 197, 259, 289), levulose (176, 197), mannit (186, 197, 237), galactose (176), sucrose (1, 24, 176, 197, 200, 237, 243, 259, 286, 289, 299), lactose (176, 186), dextrin (186), inulin (1), melamprit (186), maltose (176, 196), sorbitol (259), dulcitol (176) and perhaps humus extract (1), but not from erythrit

(176), oxalic acid (138), tartaric acid (138), tannin (138), acetic acid (138), xylose (192) or dioxyacetone (192).

Leonard (142) believed that starch could be formed in leaves from practically any sugar or sugar alcohol.

In what is perhaps the most comprehensive treatment of the subject, Nadson (186) studied starch formation from other carbohydrates in the detached destarched leaves of 19 dicotyledons, seven monocotyledons, four algae and one pteridophyte. He found that 24 out of 26 species tested formed starch from sucrose, 21 out of 22 from dextrose, seven out of 11 from lactose, 16 out of 26 from glycerine, six out of eight from dextrin, three out of 13 from mannit, and two out of three from melamprit. Only *Elodea* and onion formed no starch from any of the substances tested. Quercit, glycogen, gum arabic, calcium saccarate, tartaric acid, oxalic acid, malic acid, potassium tartrate and ammonia did not induce starch formation in any leaves tested. All materials which induced starch formation in Nadson's tests had alcohol radicals.

Next to starch, sucrose is perhaps the most important storage form of labile carbohydrates. Leonard (142, 143) found that sucrose accumulation in leaves was progressively less in leaves fed 6% sucrose, fructose, glucose, lactose, maltose and galactose, and no sucrose was formed from d'mannose, l'xylose, l'arabinose, d'sorbitol, d'mannitol or d'dulcitol. Hartt (100, 101) found that sugar-cane leaf blades absorbed large quantities of dextrose or fructose and that these were converted one into the other in the leaves, but that the concentration of each within the leaf remained low while the excess was converted to sucrose. Others, too, have studied sucrose synthesis from monosaccharides in detached leaves (150, 192, 282). Specificity of different sugars for leaves of different species are also discussed (102, 176).

The concentration of the test sugar is of course important in determining whether or not it is absorbed in detectable amounts by leaves. With sucrose solution, starch increase in the leaves proceeds parallel to sucrose concentration of the substrate (299) with a minimum concentration at about 0.1% (170, 299), an optimum concentration at about 10% but with little difference between 5% and 20% (24, 71, 78, 100, 176, 197, 286, 299, 306), and the maximum difficult to define but perhaps around 40% (78, 100, 197, 299, 306). Saposchnikoff (239) believed that the optimum sugar concentration of the substrate was about the same as the optimum sugar concentration within the leaf, and Weevers (289) found that leaves could take up sugar from hypertonic solutions. With less favorable sources of carbohydrate a higher concentration is probably necessary to produce the same effect on leaf vigor or carbohydrate storage, and Meyer (176) observed starch formation by beet leaves on 20% glycerine, but lower concentrations were generally ineffective. Glycerine was approximately equal to sucrose in another study, however (277). Leaves which in nature form little or no starch require a higher sucrose concentration or more time for starch formation in detached leaf cultures than do leaves which normally form starch (243).

For leaves with their petioles in the nutrient solutions and their laminas exposed to the prevailing atmospheric conditions, the optimum sugar concentration of the substrate is lower than for leaves floating on the test solutions in moist chambers (24, 306).

The increased length of life of detached leaves resulting from the use of sugar solution instead of water as a substrate has been noted (22, 24, 78, 159, 277, 281, 286, 306). Sucrose and dextrose are more readily utilized by leaves than are other sugars (143, 176, 197, 277, 306), and sucrose is sometimes considered more favorable than dextrose (176, 197, 277, 286, 289, 306).

Detached green and chlorotic leaves utilize sugars about equally (24, 42, 100, 237, 289, 331), but albino leaves did not form starch from glycerine or mannit (237) or from glycerine or sorbitol (259). Weevers (289) found that starch formed at lower sugar concentration in green than in chlorotic leaves. In wilting leaves starch is transformed to sucrose (2, 143, 246), and this transformation is independent of light (2) but can be prevented by killing the leaves at 72° C. before drying them (143) or by killing them with freezing, chloroform or toluene (261). A similar transformation occurs in drying potato tubers (284) and is therefore not peculiar to leaves.

Riehm (220) reported that sugar solution applied to leaves inhibited the formation of shoots but had less effect on root formation. Trelease and Trelease (277) found that chlorophyll was retained longer in leaves supplied with glycerine than in leaves supplied with the sugars tested by them. Winkler (299) and Phillis and Mason (200) noted that oxygen was necessary for the absorption and interconversion of sugars by leaves and that the process was prevented by narcotics. Hartt (100) found that arsenite or selenite added to water in which sugar-cane leaves were standing in the light, prevented the accumulation of fructose but permitted accumulation of dextrose. Nurmia (192) compared the ascorbic acid contents of leaves held on water and on sugar solution substrates, and found no difference. Klebs (124) observed that degeneration of the chloroplasts occurred when *Zygnema* was cultured in sugar solution in the light, but such has apparently not been recorded for detached leaves of higher plants.

Pucher *et al.* (210) studied changes in organic acids in detached leaf cultures of rhubarb, and Vickery *et al.* (281) point out important correlations between organic acids and carbohydrate metabolism in detached leaves. Schimper (244) found that chlorophyll-free leaves were not able to make calcium oxalate. Fats of leaves are apparently less readily utilized than are sugars, for Chibnall and Sahai (45) found no significant changes in the fats of detached leaves of Brussels sprouts cultured for eight days with their petioles in water.

The importance and utility of carbohydrates in the nutrition of leaves is excellently illustrated by Spoehr's (259) total cultivation of albino corn by supplying sugar to the cut ends of the leaves of plants growing in a mineral nutrient solution.

The relation of sugar used as a substrate to the growth of parasitic fungi on leaves and to the development of contaminating fungi on leaves or in the culture medium will be treated in later sections of this paper.

Mineral supply. Detached leaves may absorb or release salts through their normal or injured surfaces, but these functions have not been shown to be important in detached leaf cultures. Absorption of mineral salts through uninjured surfaces of detached leaves was studied as early as 1754 by Bonnet (26), and has since been demonstrated by Boehm (23), Boussingault (29) and Dandeno (53), while Schimper (243) observed increased growth of detached leaves fed salts through their cut petioles. Mineral salts are fed to attached leaves in commercial practice in some cases by spraying the salts on the plants (298). In studies with detached leaves, however, most investigators have found no advantage in adding mineral nutrients to the substrate (78, 133, 240, 277, 280, 286, 306), some have found mineral nutrients injurious through their effect in increasing contamination (133, 306), and others have used mineral nutrients for detached leaf cultures without commenting on their utility (4, 91). In the absence of sugars in the substrate, Schimper (243) found that leaves lived longer in well water than in distilled water, and longer in complete nutrient solution than in well water. Riehm (220) noted that leaves grew more rapidly in dilute mineral nutrient solution than in distilled water, but in concentrated mineral nutrient solution, root development was inhibited, and in alkaline solutions roots were formed but not shoots.

Diffusion of mineral and organic salts from detached leaves into the substrate probably occurs continuously but is more rapid when the leaves are injured from whatever cause. Unfortunately for purposes of this review, most studies of this phenomenon have been with attached leaves. Arens (7) reports that when pure water such as dew is deposited on leaves, the cuticle becomes permeable to salts and other solutes and considerable exosmosis occurs. On many plant species dew drops were alkaline and exudation drops were acid. Distilled water at pH 5.6 became alkaline in a few hours when sprayed on foliage—more alkaline on upper than on lower leaf surfaces, and more alkaline on plants in sun than on plants in shade. The alkalinity was considered due to potassium, calcium and magnesium as well as organic substances, and Arens found that the mineral content of leaves could be reduced as much as 50% in 24 hours through exosmosis. Arens considered that the process of excretion functions as a regulatory mechanism whereby plants which have absorbed too great a quantity of minerals may decrease their salt content. Other evidence of loss of mineral salts through leaves has been presented (162, 177, 283), and Wallace reported a loss of up to 30% dry matter, 60% ash and 100% per cent potassium in four leachings in four days.

Brown's (35) study of the exomosis of nutrient substances from the host to the infection drop emphasizes the importance of such materials in plant pathology.

In the usual non-sterile methods of handling detached leaf cultures, the release of mineral salts from the leaves to the substrate is undesirable because of the effect of these salts in increasing the growth of saprophytic fungi and bacteria in the substrate and on the leaves. This release of salts into the substrate is likely unavoidable, but can be reduced in quantity by avoiding injury to the leaves, by choosing vigorous leaves, and by using a substrate which will maintain the leaves in a vigorous condition. The growth of micro-organisms in the substrate made more nutritious by these mineral salts, can be further reduced by an approach to aseptic methods, and by occasional changing of the substrate solution. In aseptic leaf cultures, on the other hand, the above statements would not apply, and it is quite likely that the addition of nutrient salts to the substrate would be desirable (159, 290)

Other chemical supplements. In addition to sugars, which have been shown to have a decidedly beneficial effect on detached leaves when added to the substrate, and mineral nutrients, which are usually injurious under ordinary conditions of detached leaf cultures, there are a number of chemicals which would seem from indirect evidence to be of possible value if added to the substrate of detached leaf cultures, but which have not yet been adequately studied.

Jennison (120) reported that additions of boric acid, nitric acid, formaldehyde, potassium permanganate, charcoal and peppermint increased the life of some cut flowers. Laurie (139) found that many flowers kept longer if the receptacle contained copper metal. He also believed that hydrazene sulphate, sucrose, copper sulphate, sodium amitol and zinc powder might prolong the life of flowers. Sulfurous acid is said to prolong the life of cut bowers (6). Neff (188) studied the effect of some chemicals on the keeping quality of cut flowers, but his data are rather unsatisfactory. Hitchcock and Zimmerman (105) secured no marked improvement in the lasting qualities of several cut flowers by adding any of 50 chemicals to the water in which the stems were immersed. There was some evidence of improvement with potassium permanganate and ethyl alcohol, however.

Dawson (59, 60) found that glutamic acid, glutamic acid hydrochloride and nicotine hydrochloride added to the solution in which tobacco plants detached from their roots were standing, increased the absorption of solution, increased the life of the plants, delayed the loss of chlorophyll, delayed wilting and increased the nicotine content. The surface-active materials normally present on leaf surfaces (206) might themselves have an effect on the life of detached leaves in liquid culture. In most of the above cases it *is not clear whether the effect of the substances referred to is* due to a direct effect on the leaves or flowers, or due to their effects on the growth of contaminating organisms.

Raber's report (213) of a possible relationship between hemoglobin and chlorophyll, as shown by the use of liver extract, would suggest the use of liver extract as a means of increasing the life of detached leaves, but in the writer's unpublished tests with nonsterile materials, contamination was excessive and the life of detached bean leaflets was not increased by adding liver extract to the water or sugar solution used as a substrate for detached leaves.

Species. Important differences in the time that leaves of different species may be kept in a vigorous condition in detached leaf cultures have been noted, but few generalizations can be drawn. Leaves of dicotyledons usually live longer than leaves of monocotyledons (48, 83, 286), and this might be because (*a*) monocotyledonous leaves usually store their labile carbohydrates as soluble sugars, while starch prevails in the leaves of most dicotyledons (24, 197), (*b*) detached leaves of monocotyledons form callus and roots less readily than do leaves of dicotyledons (96, 136) and (*c*) there is greater cut and injured leaf surface when most monocotyledonous leaves are detached than when dicotyledonous leaves are detached. In spite of this, Weintraub (290) has reported greater success in the culture of oat leaves than most other investigators have reported with dicotyledons.

The hardier leaves of shrubs and trees usually remain in a vigorous condition longer than leaves of herbaceous plants (26, 48).

Contamination and infection. The surfaces of leaves and fruits as well as many other outdoor surfaces are normally heavily infested with bacteria and fungi (39, 163, 253, 262, 270). Some of these micro-organisms grow well under the conditions normally maintained in detached leaf cultures and are usually associated with the decline in vigor and eventual death of the leaves (24, 48, 59, 78, 84, 133, 159, 183, 197, 220, 286, 299, 306). Though bacteria occur in numbers of 32,000 to 2,000,000,000 vegetative cells and 20 to 200 spores per gram of leaves (39, 253), they are apparently less injurious to leaves in detached leaf cultures than are fungi, though adequate proof of this is not available. Bacteria occur in large numbers in detached leaf cultures, but they usually do not cause a visible clouding of the substrate until the leaf decays. Their occurrence and injurious effects in detached leaf cultures have been noted (279, 291), and the extensive development of bacteria in the vascular systems of detached leaves or shoots with their stems in water is believed to be partly responsible for the early wilting of these organs, but the bacteria occurring in detached leaf cultures have received little attention by most investigators. The apparent relative unimportance of bacteria, as compared to fungi, is possibly due to the acidity of the substrate (203) and its deficiency in nitrogen and other nutrients, as it has been observed that addition of nitrate increased bacterial growth in detached leaf cultures (306).

Adequate surveys of the number of fungus spores occurring on healthy leaves have not been found, though it is well known that any exposed leaf surface soon has a heavy population of fungus spores, and Marchal (163) lists about 80 species of fungi found on the surface of fruits. Which species are most important is not known, but *Penicillium, Rhizopus* and *Hormodendrum* are among the genera frequently found growing on the leaves when they start to decay. Under the abnormal conditions of detached leaf cultures, many fungi not normally considered pathogens will attack the leaves, and the distinction between true parasites and saprophytes is difficult (326).

The occurrence of fungus spores and organic matter on the leaves, the inherent vigor of the leaves, the nutritive value of the substrate, and light, are of importance in determining the development of fungus and bacterial contaminants in detached leaf cultures. Attempts to increase the length of life of detached leaves by reducing the initial population of micro-organisms have consisted of washing the leaves with water before use (48, 100, 133, 286) or with surface sterilizing substances such as mercuric chloride, alcohol, calcium hypochlorite, hydrogen peroxide and formaldehyde (78, 91, 183, 220), but only Mothes (183) indicates a definite improvement from such treatments. The reduction of established contaminants has been attempted by frequent changes of nutrient solutions (100, 183, 286, 299) and the trimming away of the cut or contaminated leaf edges (100, 183, 286). Other possibilities for reducing contamination which have been indicated but not properly explored are scalding or charring the cut ends before use (120) and the addition of bacteriostatic and fungistatic chemicals (80) to the liquid substrate. Use of cleaned and sterilized glassware and other equipment (133, 286) are procedures which can be expected to eliminate nutrients favorable to the growth of contaminants and to kill micro-organisms which might otherwise be introduced into the cultures.

The longer life of young and medium aged than of old leaves, and the reduced contamination with greenhouse-grown as compared with field-grown leaves, as observed by Dundas (78) and the writer (unpublished), are probably related in part to the differences in the numbers of fungi, bacteria and organic matter on such leaves.

A high state of leaf vigor, though a rather indefinite quantity for which no definition will be attempted here, tends to reduce the growth of contaminating organisms on leaves and in the substrate. Leaf vigor may be influenced by conditions of plant growth before detachment, by time of day of detachment, by age of leaves before and after detachment, by carbohydrate and other nutrition of the leaves, by mechanical injuries to the leaves, by infection of the leaves with pathogens, and probably by many unknown factors, and it is of course difficult to separate direct from indirect effects. Dundas (78) found that leaves injured by *Diabrotica* beetles and by other causes, and leaves from plants grown on alkaline soil, were especially prone to contamination. Meyer (176) noticed that attacks by contaminating fungi were less when starch was formed in abundance in detached leaves. Yarwood (306) believed that, in general, the growth of the obligate parasites, *Uromyces* and *Erysiphe,* was better, and the growth of the facultative saprophytes, *Colletotrichum* and *Macrosporium,* was poorer, when clover leaflets were in a high state of vigor as influenced by the time of day the leaves were detached, the age of the leaves and the sugar content of the substrate, and he believes that these generalizations would likely apply to other leaves and micro-organisms. The greater the vigor of leaves the less their permeability (271), the less their permeability the less the diffusion of nutrients from the interior of the leaf to the substrate, the less the nutrient value of the substrate the less the growth of contaminating organisms therein, the less the growth of micro-organisms in the substrate the less the chemical injury to the leaves and the less the likelihood of these organisms attacking the leaves directly.

The nutrients added to the substrate of detached leaf cultures will affect directly the growth of micro-organisms present in the cultures or may affect the growth of organisms indirectly through the effect of the nutrients on the vigor of the leaves. Of the two carbohydrate nutrients commonly used, dextrose is more favorable than sucrose to the growth of most pathogenic bacteria (36, 178, 286) or to the organisms encountered in detached leaf cultures (78, 286, 306). Even when sucrose is used as a substrate it may be hydrolyzed to dextrose and fructose by autoclaving (149, 185), by the action of acids released into the cultures by the leaves (203), or by the action of micro-organisms in the cultures (36, 272). Waters' (286) observation that routine sterilized sucrose solutions were more favorable for the growth of contaminants in leaf cultures than were unsterilized sucrose solutions, might indicate some change other than hydrolysis, for Mudge (185) and McAlpine (149) found no hydrolysis of sucrose solutions till they had been autoclaved for more than 30 minutes. In the writer's tests, however, 10% solutions of C.P. or commercial sucrose, prepared with distilled water in routine cleaned glassware and sterilized by steaming for an hour or by autoclaving for five or more minutes at 15 pounds pressure, has shown much hydrolysis, as determined with Benedict's solution or with Fehling's solution. Similar unsterilized solutions have shown no hydrolysis or only a trace of it. Sterilization of sucrose or other nutrient solutions used as substrates for detached leaves is commonly practised, but usually without recorded desirable effect (133, 183, 197), and unsterilized sucrose has been used with satisfaction (78, 286, 306).

Addition of mineral nutrients alone or of mineral nutrients plus sugar to the aqueous substrate usually increases the growth of contaminating organisms in the cultures (78, 133, 306), though mineral nutrients have been used without recorded undesirable effect (91, 159, 286, 290). Yarwood (306) observed that the addition of magnesium nitrate to detached leaf cultures on sucrose solution favored the growth of bacteria in the substrate, while calcium phosphate favored the growth of fungus contaminants.

Light inhibits the growth of many moulds and bacteria (167), and several investigators have kept their detached leaf cultures in light (48, 286, 306). Usually only diffuse light is used, for if small closed dishes are exposed to full sunlight the enclosed leaves are likely to be killed or injured by the heat (286), though no such injury occurs in open dishes.

Among the miscellaneous treatments for reducing contamination, Dawson (59) indicated that he delayed the decomposition of tobacco plants detached from their roots by chilling the culture solution to 5° to 10° C., and McGregor (153) believed that contamination could be reduced by the use of sun-sterilized water. Winkler (299) reports that in appropriate vessels containing phosphoric acid, leaves remained sterile for several days, and by frequently changing the solution, the leaf pieces on sugar solution were kept entirely free of fungi for several weeks.

The detached leaf may liberate at the cut surface, materials of a fatty nature which may be deposited as suberin in the walls of the intact cells (207). These fatty deposits inhibit penetration of the leaf by micro-organisms of decay, and the cork layer formed beneath the layer of fatty deposit is a further barrier to penetration by micro-organisms. The speed of formation of these fatty deposits may determine whether the wounded leaf decays or heals. If the cut surface is immersed in water decay is more likely than if the cut surface has access to air.

Few attempts have been made to maintain detached leaf cultures under aseptic conditions. Use of sterilized dishes and nutrients offers no special difficulty, but securing vigorous leaves free of fungi and bacteria is a major though not unsurmountable problem. By securing his leaf material from plants grown aseptically in test tubes, and by other precautions, Mains (159) was able to grow *Puccinia sorghi* on detached corn leaves apparently free of other organisms. Weintraub's (290) results in cultivating excised oat leaves in culture media containing sugar and inorganic salts are so outstanding that the writer feels that his cultures were also aseptic, though this is not specified in the abstract. The work of Bobilioff-Preisser (21) was also presumably done under aseptic conditions.

For fundamental studies of detached leaves over long periods, aseptic cultures are necessary. For the few cases where cultures can not even be maintained long enough to secure the desired results with the methods used, aseptic cultures would likely offer a solution. However, for studies where detached leaf cultures are used only as a convenient tool for other studies, as is the case with most studies of detached leaves up to the present time, and contamination does not interfere seriously, the extra labor involved in maintaining aseptic cultures would obviously not be justified.

Infection of detached leaves, like infection of attached leaves, usually reduces the length of life of the leaves. The distinction between infection and contamination made here is that infection is limited to those organisms normally pathogenic to the leaves, while contamination refers to the association of the leaves or substrate with organisms not normally pathogenic to the leaves. Dundas (71) found that detached bean leaflets infected with *Erysiphe polygoni* lived two to eight days less than non-infected leaflets. In the writer's studies several species of detached leaves artificially infected with different powdery mildews, different rusts, different downy mildews, *Phytophthora infestans, Colletotrichum trifolii, Macrosporium sarcinaeforme* and *Diplocarpon rosae* have consistently died sooner than comparable healthy leaves. No tendency of any of these organisms to grow into the nutrient substrate from the infected leaves was noticed.

Time of day of detachment and age of leaves. Leaves detached from plants toward the end of the light portion of the day have a greater content of carbohydrates (47, 51, 169, 179, 199, 226), of proteins (43, 183) and of minerals (201) than leaves detached just before the beginning of the light portion of the day. Since detachment eliminates or reduces translocation out of the leaf, it would be expected, therefore, that because of their greater amount of stored food, leaves detached in the afternoon would live longer than leaves detached in the early morning. This has been observed for apple (180) and for clover (306), though Dundas (71) noted little difference attributable to time of day of detachment for bean leaves on 10% sucrose. Hartt (101) reports that the ability of sugar-cane leaf blades to interconvert sugars varied with the time of day at which the leaves were removed from the plant, and this might indicate some diurnal variation in the enzyme system.

Young to intermediate-aged leaves usually live longer than old leaves, though there seems to be little quantitative information on this matter (78, 306).

Formation of callus and roots. The formation of callus (48) and of roots (173) is believed to increase the life of detached leaves. This is likely because of the wound healing (207) which normally precedes the formation of callus and roots.

UTILITY OF DETACHED LEAVES

Plant propagation. A large number of but probably not most species of higher plants may be reproduced by leaf cuttings. This subject has been reviewed (268) and will be discussed only briefly here. Extensive investigations of leaf cuttings have been made (96, 247, 265) and of the 1204 species tested by Hagemann, 389 formed neither roots nor shoots, 501 formed only roots, 25 formed only shoots and 289 formed both roots and shoots. Reproduction by means of leaf cuttings was more successful with dicotyledons than with monocotyledons or with gymnosperms. Hagemann considered the condition of the mother plant before leaf detachment to be important in deciding whether or not root and shoot formation would occur, and that light and nutrient conditions were relatively unimportant. Riehm (220) found that the more concentrated the nutrient solution the more limited the root development, and that alkaline solutions favored shoot formation but had no effect on root formation. According to Schwarz (247), infiltration of the leaf petiole with water or with diastase, or culture in the dark for six to eight days before transfer to light, favored root formation. Inclusion of an axillary bud or meristematic tissue on the leaf cutting makes shoot formation much more likely (87, 117). Detached leaves have the advantage over shoot cuttings that more individuals can be propagated from the same plant.

Physiology. Much of our knowledge of the physiology of leaves, which is a large phase of the physiology of higher plants, has come from studies with detached leaves. This is probably largely because of the great ease with which they can be manipulated and the results evaluated as compared with those of entire plants. Detached leaves have been successfully used in studies of water absorption (23, 26, 29, 31, 40, 53, 68, 89, 97, 104, 129, 170, 184, 278, 293, 329), of salt absorption (23, 29, 53), of transpiration (14, 16, 29, 37, 55, 56, 57, 66, 69, 86, 88, 103, 107, 112, 113, 118, 123, 131, 132, 157, 168, 181, 182, 198, 208, 225, 250, 263, 309, 319, 322, 323), of respiration (3, 4, 11, 18, 27, 28, 32, 33, 34, 61, 90, 93, 115, 116, 130, 141, 160, 164, 190, 194, 195, 205, 217, 219, 241, 242, 274, 276, 294, 303, 307, 319, 324), of photosynthesis (3, 18, 32, 33, 34, 83, 106, 113, 114, 116, 212, 216, 226, 254, 255, 256, 258, 260, 294), of carbohydrate synthesis in the absence of photosynthesis (2, 24, 25, 42, 45, 100, 101, 102, 109, 124, 138, 142, 143, 176, 186, 192, 197, 200, 237, 238, 239, 240, 243, 246, 251, 261, 281, 282, 289, 299, 331), of protein metabolism (8, 154, 177, 183, 184, 244, 266, 267, 269, 279, 280, 281, 325, 327, 328), of wound responses (11, 12, 17, 63, 135, 136, 241), of translocation within the leaf (202, 226), of transplantation (133) and of electrical phenomena of leaves (236). They could probably be used in many other studies. Roach (222) used leaf injection of attached leaves in studies of mineral deficiencies of plants, and likely such studies could be performed to advantage with detached leaves.

In most studies adequate comparison of the results of attached and detached leaves has not been made to justify the belief (90, 104) that results with detached leaves apply in general to attached leaves, though the evidence available tends to support it. Detached leaves are uniquely superior to attached leaves in tests involving (*a*) weight changes, since it is impractical to weigh attached leaves; (*b*) gas exchange, since the petiole of attached leaves offers a means of gas exchange between the leaf and the stem, and since a closed system can therefore not be obtained with attached leaves; (*c*) chemical changes in which translocation is a complicating factor; and (*d*) comparison of leaves of the same plant, as of leaves of different ages. Detached leaves are superior to attached leaves in many other studies, especially if there is reasonable assurance that the results apply in general to attached leaves. The similarity in morphology and physiology of opposite leaves or leaflets offers an opportunity of securing more comparable test units with detached leaves than is possible with entire plants.

The two principal objections to the use of detached leaves in the study of the physiology of leaves are that they can frequently not be kept in a vigorous condition long enough for the desired tests in the desired environment, and that the results secured with detached leaves might not apply to normal attached leaves about which the information is desired. No attempt will be made to minimize the importance of these objections.

Culture of plant pathogens. Detached leaf cultures have been used as a substrate for the growing from spore to spore (total culture) of members of the Peronosporaceae (189, 313), the Erysiphaceae (5, 15, 70, 71, 72, 73, 74, 76, 78, 98, 119, 155, 209, 227, 228, 229, 230, 231, 232, 233, 234, 248, 277, 305, 306, 307, 308, 310, 311, 315), the Uredinales (9, 10, 22, 48, 75, 77, 79, 84, 91, 159, 180, 264, 275, 285, 286, 306, 307, 312, 316), the Sphaeropsidales (306), the Melanconiales (20, 296, 306), the Moniliales (82, 174, 306) and also for the more limited culture of fungi where the extent of growth was less or was not clearly indicated (3, 4, 19, 35, 46, 49, 67, 85, 110, 164, 166, 190, 193, 203, 205, 214, 216, 217, 252, 291).

Purdy (211), Takahashi (269) and Woods (301, 302) have demonstrated multiplication or increase of tobacco mosaic in detached leaves inoculated after detachment. Purdy observed inclusion bodies but no macroscopic symptoms, while Woods secured clear macroscopic symptoms as well as inclusion bodies in leaves of *Nicotiana glutinosa.* In most of the above cases, the fungi have been primarily leaf parasites, but *Cicinnobolus cesatii,* parasite of powdery mildews, was cultured on powdery mildews which were in turn cultured on detached leaves (306).

Detached leaves have apparently not been used extensively for the culture of phytopathogenic bacteria, but Dr. P. A. Ark (unpublished) informs the writer that he has successfully used detached leaves for the culture of *Phytomonas pisi, P. phaseoli* and others. Detached leaves have undoubtedly been used, without specific mention in publication, in inoculations with many other organisms parasitic on plants.

In considering more specific types of plant disease problems, detached leaves or shoots have been used in studies of leaf penetration (19, 35, 49, 189, 235, 252, 316), comparative susceptibility of leaves to inoculation through upper and lower surfaces (20, 48, 286, 316), induced susceptibility (230, 233), effect of environment on susceptibility (15, 20, 48, 78, 85), effect of vitamins on susceptibility (209), effect of age of tissues on susceptibility (78, 306), effect of carbohydrate nutrition on susceptibility (78, 159, 203, 277, 285, 286, 306), host ranges and varietal susceptibility (48, 67, 70, 71, 72, 73, 74, 75, 76, 79, 98, 147, 180, 189, 227, 231, 248, 275, 310, 312), susceptibility of different leaf tissues (155, 230, 233), nature of resistance (155), inheritance of resistance (71, 72, 75, 76, 77, 310), comparative infective powers of different spore stages (48, 227), heterothallism of fungi (180, 308), formation of perfect or telial stages (22, 214, 285, 286, 308), occurrence of physiologic races of pathogens (71, 73, 74, 78, 79, 119, 227, 228, 232, 234, 275, 310, 312, 315), formation of toxins by pathogenic fungi (291, 330), transpiration changes induced by infection (103, 309), respiratory changes induced by infection (3, 4, 141, 164, 190, 205, 217, 307, 319), photosynthesis changes induced by infection (3, 216), overwintering (231), diurnal cycles of pathogenic fungi (46, 309, 314), protective fungicides (110, 166, 313) and volatile fungicides (317).

Determination of the conditions governing the formation of teliospores by rusts is a good example of a problem in which detached leaf cultures played an important part in the solution. By manipulating the sugar nutrition of his cultures of several species of rusts on detached leaves, Waters (286) was able to demonstrate quite clearly that teliospore formation was usually stimulated by a decline in the amount of carbohydrates available to the leaves. That this principle was not universally applicable was apparent from Waters's observation that teliospore formation of *Puccinia triticina* was apparently favored by high carbohydrate nutrition of the leaves, an observation later confirmed by Bockstahler (22), also using detached leaves.

The most extensive practical use of detached leaf culture known to the writer has been made by Dundas (70, 71, 72, 73, 74, 75, 76, 77, 78, 79) who has maintained many physiologic races of bean powdery mildew (*Erysiphe polygoni*) and of bean rust (*Uromyces appendiculatus*) on detached leaves for several years and has tested the response of bean varieties, strains and segregating progenies to these many races of the two above pathogens in thousands of detached leaf culture tests. Such studies, with *Erysiphe polygoni* at least, would have been difficult or impossible by inoculations on entire plants.

*Studies of the comparative development of pathogens on de*tached leaves and on entire plants indicate that in most cases the two methods give similar results in regard to susceptibility of hosts and virulence of pathogens, though adequate comparisons have apparently been made only with powdery mildews and with rusts. Clinton and McCormick (48) found that with aeciospores of *Cronartium ribicola*, 66% of 170 inoculations on detached leaves and 78% of 123 inoculations on potted plants were successful. With uredospores of *C. ribicola*, 57% of 169 inoculations on detached leaves and 57% of 54 inoculations on potted plants were successful. The ratings of disease intensity, while not clearly defined, were usually less for detached than for attached leaves, however. Clinton and McCormick's tests on the detached leaves were made with water as a substrate, and it is to be expected that if sugar solution had been used, the rust development would have been greater on the detached leaves.

Fawcett (85) found that the maximum temperature for infection of citrus leaves with *Cladosporium* was higher for detached than for attached leaves.

Blodgett (20) concluded that inoculations of *Pseudopeziza* on detached *Ribes* leaves in moist dishes proved to be as reliable as those on potted plants.

Waters (285, 286) found that infection of rusts on detached leaves was sometimes greater than on potted plants, and that while it was difficult to maintain some rusts on potted plants during the short days of winter, it was relatively easy on detached leaves on sugar solution.

Dundas has shown a satisfactory correlation in the susceptibility of beans to powdery mildew (71) and rust (79) on detached leaflets and on field or greenhouse plants, but has observed that powdery mildew sometimes grew more luxuriantly on detached than on attached leaves (74).

Newton and Yarwood (189) reported that detached and attached leaves were equally susceptible to hop downy mildew.

Powdery mildews, like rusts, may develop poorly on greenhouse-grown plants during the winter months. To compare the growth of powdery mildew on detached and attached clover leaves, the writer (305) heavily inoculated leaves of six plants of red clover of varying susceptibility on January 10, allowed two leaflets of each leaf to remain on the plant and transferred one leaflet of each leaf to 10% sucrose in a Syracuse watch glass. The Syracuse watch glasses and potted plants were kept in the same greenhouse environment. After 11 days the powdery mildew showed luxuriant development on all detached leaves but relatively poor or no development on the attached leaflets. In sunny summer weather development on attached leaves was usually approximately equal to that on detached leaves. In tests of the host range of powdery mildew on *Trifolium* species, Yarwood (310) secured infection on detached leaves of some species on which no infection was detected on plants in the field or inoculated plants in the greenhouse.

Carbohydrate nutrition of leaves is apparently more important in the culture on detached leaves of the obligate parasites, the powdery mildews and rusts, than in the culture of other fungi pathogenic on such leaves. Salmon (227, 228, 229, 230, 231, 232, 233, 234, 235) and Clinton and McCormick (48) report good infection of powdery mildews and of rusts, respectively, without addition of carbohydrates to the substrate on which their detached leaves were cultured, but it was not until other work (159, 277, 286) with sugar solutions as substrata that the potentialities of detached leaves for the culture of these two important groups of obligate parasites became apparent. While the pioneer work was done by Mains, the most comprehensive treatment of the subject to date is by Trelease and Trelease. In their tests wheat powdery mildew (*Erysiphe graminis*) produced 100 or more times as many conidia when detached leaves, previously carbohydrate-starved and inoculated, were floated in the dark on arabinose, xylose, rhamnose, dextrose, levulose, galactose, mannose, sucrose, maltose, lactose, melizitose, starch, dextrin, inulin, glycerine or mannite, than when similar leaves were floated on water. The best mildew development occurred on leaves floated on dextrose, levulose, sucrose or melizitose. Sucrose solution is now the standard substrate for detached leaves used to culture parasitic fungi (70, 71, 72, 119, 174, 180, 203, 209, 275, 285, 286, 305, 306, 307, 308, 310, 312).

Except for the process of inoculation, the methods of culture of pathogens on detached leaves do not usually differ markedly from the methods for the culture of detached leaves where no pathogen is involved. Inoculation methods vary with investigators and according to the organism studied, but are usually basically the same as inoculation methods used on entire plants (70, 71, 159, 180, 189, 209, 227, 275, 305, 306, 307, 310). The advantages of the use of detached leaves over the use of entire plants for cultures of parasitic fungi have been pointed out (48, 306) but will be indicated here. Some are as follows:

(*a*) Economy of space. A series of inoculations involving several strains of different species of organisms on a series of different host plants may be incubated in a few cubic inches whereas similar tests with entire plants would require perhaps 100 times as much space.

(*b*) Economy of host material. Leaves of a single plant may be distributed into many separate but closely comparable test units without sacrificing the original plant or impairing its further growth.

(*c*) Economy of inoculum. Inoculations may be made with single spores under ideal conditions for infection.

(*d*) Ease and exactness of observation. The growth of an organism such as a powdery mildew may be followed microscopically from a single conidium through conidium formation to perithecium formation without disturbing the culture or impairing its growth.

(*e*) Reduced danger from contamination. Different but microscopically indistinguishable strains of a single species may be kept effectively isolated and yet grown under comparable conditions in the same environment. This is especially important with wind-blown fungi, such as powdery mildews, which increase rapidly under the dry air conditions prevailing in greenhouses.

(*f*) Uniformity of experimental units. Opposite leaves or half leaves which are about the most nearly identical leaf units obtainable, may be quantitatively compared under different treatments with reasonable assurance that original variation between experimental units is very small, and this may be further reduced to insignificant proportions by replication. With naturally cross fertilized and heterozygous plants, such as red clover, genetically uniform experimental plants can be secured only by slow and cumbersome vegetative propagation, while a large number of genetically comparable units may be secured from a single plant by the detached leaf culture technique.

(*g*) Ease of control and manipulation of environmental factors. The effect of temperature on a leaf disease may be studied in standard laboratory incubators, whereas a similar study with entire plants would usually involve special and expensive equipment.

(*h*) Elimination of the necessity of light. Detached leaves on sugar solution can usually be kept in a vigorous state for two weeks or more in darkness, whereas entire plants, because of continued translocation in the absence of renewed carbohydrate supply, soon become deprived of stored food and die in darkness.

(*i*) More luxuriant growth of some parasitic fungi. Obligate parasites, such as powdery mildews and rusts, usually grow luxuriantly at any time of the year on detached leaves supplied with sugar, while growth on inoculated leaves on entire plants during the short cloudy days of winter is sometimes much less vigorous.

In spite of these advantages and perhaps others not recognized, detached leaf cultures are admittedly unsuitable for cultures of certain leaf parasites. Some of the situations where detached leaves have not been successful or have not served any good purpose in the writer's experience are:

(*a*) Culture of onion downy mildew. Detached onion leaves usually died in about four days, a period too short for the satisfactory culture of almost any pathogen. Penetration of onion by *Peronospora destructor* was readily followed, however, on detached leaves.

(*b*) Cultures of grape powdery mildew. Inoculations of detached grape leaves with conidia of *Uncinula necator* have usually resulted in poor development of the fungus, though only a few trials have been made.

(*c*) Routine tests of protective and eradicant fungicides and tests of the host range of a single strain of a pathogen. Tests of this type usually involve less work when performed with entire plants and yield as much information as tests on detached leaves.

(*d*) Where multiplication of the pathogen is the principal objective, unless numerous strains must be kept separate.

In addition to the above specific and generalized examples there are undoubtedly many other cases where detached leaves offer no advantage over conventional methods.

Rearing of insects. Aphids, thrips and mites, and perhaps many other insects and animals, can be conveniently kept alive or reared on detached leaves. Thrips have been so cultured (13, 81, 99, 288, 320), as well as aphids (94, 196, 321), mites (221), psyllids (140, 218) and a parasite of leaf hoppers (152). Howard (111) early pointed out the difficulties of rearing insects under natural conditions and on entire plants, and the advantages of using detached leaves, but did not establish the utility of his suggestion. Griswold (94) pointed out that aphids remain in one position on leaves for long periods and are therefore more easily adapted to rearing on detached leaves than are some other insects. He found a shorter maturation period for geranium aphids on detached leaves than on entire plants. In the writer's tests of culture of hop and clover aphids on detached leaves (321, and unpublished), many of the aphids have fallen from the leaves or crawled off the edge of the leaves and died in the substrate where they were overrun by bacteria and fungi. This objection, which is not serious enough to preclude the use of the method in all cases, is overcome by isolation of the lamina from the substrate, as described for thrips (13). Thrips are much more active than aphids and therefore more difficult to keep confined, and would not deposit eggs on parts of tobacco leaves (108), but can be maintained in adequate populations with or without special precautions to keep them confined to the lamina of the leaf. The cannibalism displayed by some insects makes culture in large numbers difficult, and this objection can be overcome in part by rearing individual insects separately.

It seems to the writer that there are many ways other than those mentioned where detached leaves could be used advantageously in the study of insects and related forms. Red spiders, for example, have laid eggs, hatched from eggs and increased by large numbers on detached clover leaflets on which clover powdery mildew was being studied. Detached leaves would offer opportunities for study of insecticides under conditions where the same insects could be kept under continuous microscopic observation on leaves without being mechanically disturbed, and would be especially useful in studies of volatile insecticides.

SUMMARY AND CONCLUSIONS

Unlike detached roots, detached leaves have not yet been induced to grow indefinitely, but individual leaves have been kept living for periods up to six years, in some cases longer than the normal life of attached leaves. Under the best conditions known, most detached leaves can be kept in good condition for about three weeks, which period is long enough for most physiologic studies. They carry on most of the functions of normal attached leaves including transpiration, respiration, photosynthesis and protein synthesis, are more easily subjected to detailed experimental manipulation than are entire plants, and much of our fundamental knowledge of leaf function has been derived from studies of detached leaves. Food materials of detached leaves do not escape in important amounts through the cut petioles, and detached leaves are therefore very suitable for studies in which it is desired to eliminate translocation as a complicating factor. By culture of detached leaves in darkness, both photosynthesis and translocation are eliminated, and carbohydrate transformations can be advantageously studied. The floating of leaves on unsterilized solutions of commercial cane sugar is now the most common method of culture of detached leaves, but minor changes are desired for different species and types of tests. Detached leaves have served as a convenient substrate for the total culture of plant pathogenic micro-organisms, especially the obligately parasitic powdery mildews and rusts. Among the more important aspects of these two important groups of parasites which have been successfully studied by means of cultures on detached leaves are carbohydrate nutrition, effect of environment on disease development, host range, physiologic specialization, heterothallism, formation of overwintering stages, respiration and volatile fungicides. Use of detached leaves as a substrate for the study of insects is a relatively unexplored field which would seem to have advantages over conventional methods in certain studies.

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THE BOTANICAL REVIEW

VOL. XII FEBRUARY, 1946 No. 2

SOME COMPARISONS OF BACTERIAL PLANT GALLS AND OF THEIR CAUSAL AGENTS[1]

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INTRODUCTION

Pathological growths on plants arise from stimulation by various agencies, and many of them are so harmful that they are commonly classed as diseases. Among the agents causing harmful galls are insects, nematodes, fungi, bacteria and various non-parasitic factors. In the present paper the discussion is limited to pathological growths caused by pathogenic bacteria and to certain similar overgrowths caused by non-parasitic agencies. Consideration is omitted both of the physiology of crown gall proper, because comparable studies have not been made of other galls, and of legume root nodules, because they are beneficial (*cf.* 20).

Bacterial galls have been known to plant workers for many centuries. The causal relation of bacteria, however, was not clearly established until Erwin F. Smith and his co-workers published their classic early work on crown gall (80, 81). This is the best known of the bacterial galls. Since that time various other gall-inducing micro-organisms have been described from different localities.

The geographic region where these bacteria first appeared is by no means certain. Doubtless they have been spread all over the world as a result of plant shipments, an obvious means of dissemination. Some investigators (*e.g.*, *7*) have considered that crown gall, at least, because of its natural occurrence in isolated cactus forests, was indigenous to the Southwest, and others, including the present senior writer, have observed crown gall on susceptible hosts in other areas which were never cultivated.

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[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by the International Cancer Research Foundation and by the Wisconsin Alumni Research Foundation. The writers are indebted to Mr. C. S. Hean for assistance in securing many of the papers cited.

Since this manuscript was prepared, a virus that causes growth has been described: L. M. Black, A virus tumor disease of plants. Am. Jour. Bot. 32: 408–415. 1945.

The damage caused by these galls is greatest on susceptible crops which have been grown intensively in the same field. At one time piece-root-grafted nursery apple trees had graft knots (including crown gall, hairy root and wound overgrowths) to such an extent that a third of the susceptible trees had to be destroyed. Repetition of such loss has been prevented by improved methods of making and wrapping the grafts (*e.g.*, 49, 64, 65, 66). Crown gall on sugar beets has been quite destructive (92) unless at least a two-year rotation was employed. It is still a problem in widely scattered areas on stone fruit trees.[2] On cane fruits crown gall may be serious (1), but cane gall (29) is much less damaging. The two may be easily confused. Olive knot has been quite harmful on certain varieties of olive (98). An estimate of the economic importance of these and of other galls may be found in the papers in which the bacteria are described.

Important as these galls are on agricultural crops, a study of them seems to have value also from the standpoint of the basic phenomena involved in pathological cell growth. The fundamental problem, whether in plants or animals, has been well expressed by Szent-Györgyi (90) as follows:

> "Biochemistry teaches us that many constituents of our body are found with equal frequency in plants and animals, fulfilling analogous functions in each. These substances of plants, just as they are, or with little alteration, fit into the machinery of our cells. Two machines, the parts of which are interchangeable, cannot be very different and so anything we learn about the plant will lead us closer to the understanding of ourselves. The plant, as an object of the study of life, has, compared to man, some very great advantages. . . . The plant . . . can dispense with many of the unessential complications found in our body which enable us to walk, hear, see, smell, and think. Life in the plant will present itself in much simpler forms, and thus allow the great fundamental principles to come to the fore".

Some of the advantages provided by plants for fundamental studies in connection with galls may be listed (*cf.* 58) as follows:

(*a*) Large numbers are easily available. The number used, whether ten, a hundred or a thousand, is commonly adjusted to the statistical needs of an experiment.

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[2] Since this manuscript was prepared, J. G. Brown (Science **100**: 528. 1944) has reported the control of crown gall with a penicillin preparation. Among the chemical treatments of galls, those suggested by P. A. Ark (Blue Anchor **19**(1): 16–19. 1942) appear promising.

(*b*) The initial cost and the expense of maintaining plants are very low in comparison with those of animals.

(*c*) The species of plants attacked frequently contain varieties or selections possessing several degrees of resistance and susceptibility.

(*d*) Plants are suited to a wide range of experimental procedures, many of which are not feasible with animals.

(*e*) Epidemics caused by micro-organisms are induced with relative ease and can be studied without concern for the health of the technicians or the public. Likewise, non-parasitic but pathological growths occur spontaneously or as a result of physiological disturbances.

(*f*) The genetic purity of the host can be assured. Seed from long lines of successively self-fertilized parents are already available in many varieties of plants. When this is not sufficient, one can commonly find, or develop, experimental units all genetically identical through vegetative propagation. For example, within a named variety of many fruits and ornamentals, the numerous individuals are all vegetative parts of one originally selected parent. Except for occasional bud variations, they are all genetically the same. This is particularly advantageous in studies of disease and resistance, since pathogenicity is necessarily defined in terms of susceptibility of the host.

(*g*) Plant tissue can be cultivated *in vitro* on a medium containing only constituents of known chemical composition (reviewed, 95). This enables the plant worker to make various studies with accurate controls that to date are not possible with animal tissues.

The obvious possibilities with plant materials led Dr. James Ewing (personal communication), the well known cancer pathologist, to suggest in 1908 to Erwin F. Smith that a fundamental study of pathological growth be made with crown gall. The possible contribution to the cancer problem first inspired Smith to a long series of well known and monumental works, and subsequently stimulated a large number of other workers all over the world.

Smith did not hesitate to use various medical terms and described his different kinds of crown gall as one or another type of malignant tumor or cancer. This attracted much attention and support to his work. Various medical men praised him, while others, including Dr. Ewing, criticized him, saying that his terminology was inaccurate and therefore misleading and distracting from the real problem. Smith's usage of medical terms has been more or less followed by various more recent workers (*e.g.*, 40, 96) with rather similar praise and criticism. The crux of the matter seems to center about the definition of a cancer, a term which is clear enough in many medical cases, but which is difficult to delimit. Thus, what is or is not a cancer depends on the expert consulted. But, after all, the physiological mechanism of a pathological growth is not changed, whether it is called by one name or another. The real importance attaches to accurate experiments and clear thinking about their interpretation so as to understand these pathological cells.

Medical terms have been studiously avoided in this paper with the hope of evading the popular reports that follow any research with a connection to cancer, of avoiding fruitless discussions over definitions, of clarifying without glamour the comparisons of different kinds of bacterial plant galls as well as their causal agents, and of assisting readers unfamiliar with the problem and its implications to an accurate comprehension of the situation.

Much of the earlier literature on crown gall has been already reviewed (62), and the relations between pathological growths in plants and animals have been discussed in many well known works (*e.g.*, 40, 43, 53, 79).

In the present paper we have tried to compare the different kinds of bacterial galls as well as their causal agents and to amplify any well defined characters in which either galls or bacteria are distinctly different or prominently similar. Such a study has promise because any similarity between their biochemistry and physiology might indicate factors important in the ultimate causal relation between the pathogen and the host. Likewise, variation in character might indicate that such characters have less promise of a causal relation. While such a consideration may be based on the concept that the fundamental stimulus is more or less similar in these different types of galls, it does not necessarily follow that this is the true situation. However, it seems obvious such working hypotheses have value. We have tried also to compare with the bacterial gall some similar but non-parasitic growths that may assist in clarifying some of the factors involved.

Concerning the physiology of crown gall and of crown-gall bacteria, there is an extensive literature covered by earlier reviews (40, 62). However, so relatively little is known about the physiology of the other bacterial galls, that the physiology of crown gall has been reserved for later consideration elsewhere. Occasional important papers have doubtless been missed, and many of the less important or less representative citations have deliberately been omitted.

DIFFERENT KINDS OF BACTERIAL GALLS

The more prominent bacterial growths, together with their causal agents and more common hosts, are listed in Table 1. The wide host range of the crown-gall bacteria is continually growing beyond our former understanding of it (45). Out of 101 species tested belonging to 32 families, only 21 species failed to show infection. Montemartini's (48) list of hosts is perhaps the most complete. Doubtless it could be enlarged by improved inoculation technique on other hosts. The senior writer (unpublished) once inoculated many local Wisconsin weeds and secured galls on practically all the dicots except those having strongly acid sap. It is striking that the crown-gall organism has a wide host range, while those of

TABLE 1

SUMMARY OF BACTERIAL GALL DISEASES, CAUSAL AGENTS AND HOST PLANTS

Disease	Causal organism	Prominent hosts
Beet pocket rot	*Phytomonas beticola* (Smith, Brown, and Townsend) Bergey *et al.*	Sugar and garden beet varieties, (9)
Cane gall	*Phytomonas rubi* Hildebrand	Raspberries and blackberries, (29)
Crown gall	*Phytomonas tumefaciens* (Sm. & Town.) Bergey *et al.*	Very wide range, esp. on Rosaceous species, (45, 48)
Douglas fir gall	*Phytomonas pseudotsugae* (Hansen and Smith) Bergey *et al.*	Douglas spruce, (24)
Gypsophila gall	*Phytomonas gypsophilae* (Brown) Magrou	Baby's breath, (10)
Hairy root	*Phytomonas rhizogenes* Riker *et al.*	Apple, rose, (61)
Oleander knot	*Phytomonas tonelliana* (Ferraris) Adams and Pugsley	Oleander and olive, (75)
Olive knot	*Phytomonas savastanoi* (E. F. S.) Bergey *et al.*	Olive and ash, (73)
Pea fasciation	*Phytomonas fasciens* Tilford	Sweet pea, garden pea, petunia, geranium, tobacco, (91)

other gall-inducing bacteria are quite limited. This suggests a relatively broad base for the biological phenomena which are responsible for crown-gall development.

Resistance to crown gall is well known. Different kinds of Malling apple root stock have shown (25) characters from susceptible (No. II) to highly resistant (No. XVI). Similar resistance in *Prunus* stock has been determined (72, 74).

Host specificity for some strains of crown gall has been indicated. For example, crown-gall bacteria were isolated from naturally occurring galls on asparagus, bean and various other plants, and all the cultures obtained were pathogenic over a wide range, except that from bean, which infected only bean and none of the other plants, including *Datura, Pelargonium, Solanum* and *Helianthus* (83, 84, 86). English cultures (100) have varied in their ability to infect certain hosts. Host specificity in one strain from hops and another from walnut has also been found (47). These strains were actively pathogenic on tomato, sunflower and *Bryophyllum,* for example, but produced no or only a slightly pathogenic reaction on Paris daisy.

The comparative pathogenicities of *Phytomonas tumefaciens, P. beticola, P. savastanoi, P. tonelliana* and *P. rubi* were examined by cross inoculations into their respective hosts. Every organism, except *P. tumefaciens* which induced galls on all species used (52), was pathogenic on its own host but was non-pathogenic on the principal host of the other organisms.

COMPARISON OF BACTERIOLOGICAL CHARACTERS

Some characters of the various bacteria are compared in Table 2, according to Elliott's (19) procedure. Some reservations are necessary about these comparisons. Pinckard's work (52) was the only instance found in which a group of the organisms was used simultaneously. Since results secured by different workers have not always agreed, the seemingly best descriptions were used in compiling the table. Descriptions are available of other characters possessed by some of these bacteria (26, 68).

These nine gall-forming bacteria (Table 2) have the following characters in common, which are omitted from the table: all are small rods approaching one another in size, are not spore-formers and are not acid fast; many of them form chains under favorable

TABLE 2
Comparisons between Characters of Various Cell-Stimulating Bacteria

ORGANISM	OXYGEN		GELATIN LIQUEFACTION	NITRATE REDUCTION	CHROMO-GENESIS	REACTION FROM CARBON SOURCES								VEGETATIVE CELLS					MILK		INDOL	HYDROGEN SULPHIDE	AMMONIA	THERMAL DEATH POINT
						DEXTROSE		LACTOSE		SUCROSE		GLYCERINE												
	AEROBE	FACULTATIVE ANAEROBE				ACID	ALKALIN	ACID	ALKALIN	ACID	ALKALIN	ACID	ALKALIN	LENGTH (MICRONS)	DIAMETER (MICRONS)	CHAINS	GRAM	CAPSULES	CURD	PEPTONIZATION				
PHYTOMONAS TUMEFACIENS (61, 52, 87)	+		O	O	WHITE	T	O	T	O	O	+	+	O	75-2.25	0 3-1.0		O	+			O		+	52°C.
PHYTOMONAS RHIZOGENES (61, 87)	+			O	WHITE	+	O	+	O	T	O			0 5-2 6	0 1-0 8		O	+			O		+	52°C.
PHYTOMONAS SAVASTANOI (76, 52, 78)	+		O	O	WHITE	+	O	O	+	O	+	T	O	1 2-3 0	0 4-0 8	+	O		O		+		?	43°C.-46°C.
PHYTOMONAS TONELLIANA (75, 52)	+		O		WHITE	+	O	O	+	O	+	+	O	1 5-2.5	0 5-0.6			O	O		+		?	51°C.
PHYTOMONAS BETICOLA (9, 52)	+		+	+	YELLOW	+	O	+	O	+	O	+	O	0 6-2 0	0.4-0 8	+	+ O	+	+		O	+	+	51°C.-52°C.
PHYTOMONAS FASCIENS (91)	+		O	+	YELLOW	+	O	O	O	+	O	+	O	1 5-4 0	0 5-0.9	+	+	?	O		O	+	+	55°C.-57°C.
PHYTOMONAS GYPSOPHILAE (10)	+	+	+	+	WHITE, LATER YELLOW	+	O	O	O	+	O	+	O	0 4-1 2	0 2-0.8	+	O	+	+	+	O	T	+	52°C.-53°C.
PHYTOMONAS RUBI (29, 52)	+	+ WEAK	O	O	WHITE	+	O	+	O	O	O	O	O	1.72	0.64	+	O		+		O	O	+	56°C.
PHYTOMONAS PSEUDOTSUGAE (24)	+	+	+	+	WHITE	+	O	O		O		O		1.9-3 9	0 5-1.5		O					+	O	

+ MEANS POSITIVE, O MEANS NEGATIVE, T MEANS TRACE conditions. No gas was detected from nitrate and none with Smith tubes from fermented carbohydrate.

Bacterial organisms, other than those listed, sometimes have been described as gall-forming; *e.g.*, the root-nodule bacteria; *Pseudomonas pini* (16), an organism associated with a gall on pine trees; and undescribed bacteria (13) found in galls on certain forms of algae belong to the Florideae. The first of these are omitted from the list because root-nodule bacteria are beneficial rather than harmful, and the literature about them has been reviewed elsewhere (12, 20, 99). The evidence for pathogenicity of the pine-gall organism is incomplete (19), and no adequate description was found of the algal gall-formers.

The morphology of crown-gall bacteria has received special study, and a morphological irregularity has been observed in them (41, 43). In 48-hour-old cultures of the hop strain, there were Y-shaped cells and a few scattered coccoid bodies. Some of the cells had a beaded effect and others a capsule-like covering. In some cases the rods appeared to be arranged in irregular form, centering about small granular bodies. In 7- to 10-day-old cultures the rod fragments gave coccoid bodies and ghost cells. After 19 days the slime-like substance contained numerous coccoid bodies which were considered to be physiological spore-like bodies. Dry 90-day-old cultures, upon transfer to fresh media, resumed growth showing the characteristic rod-shaped forms. Others (69) have found no "Y" or warty forms. Irregular forms by *P. gypsophilae* (10), *P. beticola* (9) and *P. fasciens* (37, 91) have been observed.

A filterable stage in the life cycle has been suggested or claimed for several of these bacteria. This has been based upon experiments in which they were recovered from a filtrate. However, as Zinsser (101) has explained in detail, this does not constitute a filterable phase of a cycle. Since his postulates for demonstrating such a cycle have been fulfilled with none of these bacteria, such suggestions may be considered with reservation.

The common star-shaped aggregates in liquid media, like those from which *Radiobacter* derived its name, have suggested (85) fusion. This interesting concept deserves further study, especially with an electronic microscope and biochemical tests, to establish the character of the central granule and to rule out other possibilities, such as tangled flagellae or attraction by a particle with an electric charge different from that of the bacteria.

In physiological characters Table 2 shows various similarities and differences with perhaps one or two clues regarding pathogenicity. None of the bacteria was observed to produce gas from nitrate or from sugars without special study. With better technique all these cultures would probably produce some gas from sugars, as *P. tumefaciens* did (15). All produced some acid from dextrose, although at least with *P. tumefaciens* this probably came only from dissolved carbon dioxide. Ammonia production was of special interest because it was associated with the pathogenicity of *P. tumefaciens* almost three decades ago (77). Although present-day methods might question this early technique, there is no doubt that this and the hairy-root organism do produce ammonia (14, 97). Likewise all the other gall-inducing bacteria have been reported to produce ammonia except *P. savastanoi, P. tonelliana* and *P. pseudotsuga.* With the first two, litmus milk and certain peptone with sugar media developed an alkaline reaction suggesting ammonia production (75, 78). Since this is such a common character and notwithstanding the negative report, *P. pseudotsugae* would probably show it if studied with suitable technique. This comes near to being a common character associated with gall production.

There are other items which have been discussed (59) in relation to the pathogenicity of crown-gall bacteria, but concerning which much less is known in relation to the other gall-forming bacteria. For example: (*a*) *P. tumefaciens, P. rhizogenes* and *B. radiobacter* all reduced the oxidation-reduction potentials in several media containing plant extracts, suggesting that such bacteria growing in injured tissue might induce an "oxygen hunger". (*b*) The pathogenicity of crown-gall bacteria was destroyed by successive transfers in a dozen amino acids. Apparently this is the first instance where a pathogenic bacterium has been attenuated by a natural host constituent for which the chemical formula is well known. These studies have emphasized the importance of nitrogen metabolism in relation to pathogenesis. (*c*) All the gall bacteria apparently produce more or less gum-like material in culture. Such material from crown-gall bacteria has been identified as a polysaccharide containing approximately 22 anhydroglucose units per molecule. Such a diffusable and hygroscopic substance might easily disturb the osmotic relations of invaded tissue. (*d*) In addition to the ammonia produced by almost, if not all, gall bacteria, *P. tumefaciens* produced phosphatides, phospholipids and other substances that might "irritate" neighboring cells. (*e*) In addition, it produced various enzymes, growth substances and vitamins, such as thiamin, riboflavin, pantothenic acid and biotin. These and other factors have influenced the distribution and availability of food materials.

An unusually promising working hypothesis has been developed as a result of these studies. There have been many explanations for the cause of pathological growth, depending on this or that item (reviewed, 62). However, the first working hypothesis found in either plant or animal literature that emphasizes the importance of a suitable balance between critical items was expressed (59) as follows:

"Among these factors, as we have already seen, may be included 'oxygen hunger', changes in osmotic pressure, rearranged amounts of growth substances and vitamins, 'irritating' substances, and altered amounts of food materials. Any living cell, even a resting cell, that fails to react under such conditions seems very unresponsive.

"While we shall continue to analyze individual factors that by their presence or absence may change normal into pathological growth, there is another possibility that deserves consideration. This is that in normal growth a number of factors may operate in suitable balance. However, in pathological growth of one kind a group of these factors may be out of balance. Likewise, in pathological growth of another kind the balance is disturbed in some other way".

The literature on the physiology of crown-gall bacteria extends far beyond that of other gall organisms, and so its consideration is omitted in favor of comparisons between host-parasite interactions.

COMPARATIVE LIFE HISTORIES RE PATHOGENESIS

The pathological relations between host and parasite seem largely analogous in regard to entrance into the host, position in the host cells, exit from the host, and distribution to the new host. This approach is adapted from that devised (82) for work with animal pathogens. The situation is summarized in Table 3.

Entrance in almost every case reported in the foregoing tabulation was dependent on one or another type of wound, which varied according to circumstances. For example, in olive knot the entrance was through leaf scars, which were most susceptible immediately after leaf fall. This mode of entrance was less and less favorable with progressing time, and the infection court was closed completely by the ninth day (27). Infection was also accomplished through natural fissures in the bark (33). Oleander was successfully inoculated by spraying the pistils (75). In crown gall on red raspberry, the bacteria entered through wounds to the roots caused sometimes by cultivation but more frequently by root-feeding

TABLE 3

COMPARISONS OF SOME CRITICAL POINTS IN THE LIFE HISTORIES OF VARIOUS CELL-STIMULATING BACTERIA IN RELATION TO THEIR PATHOGENESIS[3]

Causal organism and authorities	Entrance	Primary location	Exit	Distribution
Phytomonas tumefaciens (1, 54, 63)	Wounds	Intercellular	From surface	Nursery stock, insects, cultural operations
Phytomonas rhizogenes (28)	Wounds	Intercellular	From surface	Nursery stock, insects, cultural operations
Phytomonas savastanoi (27, 33, 76, 98)	Wounds, leaf scars	Intercellular	From surface	Nursery stock, pruners
Phytomonas tonelliana (75)	Wounds	Intercellular	....	Nursery stock, insects (?)
Phytomonas beticola (9, 17, 18)	Wounds	Intercellular	From bacterial pockets to surface	Debris from infected galls
Phytomonas fasciens (37, 38, 39, 91)	Not dependent on wounds	Intercellular	From surface	Seed-borne
Phytomonas gypsophilae (10)	Wounds	Intercellular, Intracellular in water-soaked areas	... ..	Nursery stock, soapwart weed
Phytomonas rubi (2, 29)	Wounds	Intercellular	From surface	Insects, pruners, cultural operations
Phytomonas pseudotsugae (24)	Wounds	Intercellular	....	Insects

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[3] Various important and sometimes modifying details are given in the text.

arthropods, such as click-beetle larvae, millepedes and white grubs (1). The type of wound apparently had little if any effect upon the character of the overgrowth, which was determined primarily by the species of infecting bacteria; but it did influence the percentage of wounds that became infected, the size of the infection and the rate of development. Various authors (30, 54) have found that the size of the wound influences the size of the developing crown-gall or hairy-root overgrowth. Although infection by fasciation bacteria on sweet peas was reported (37) not to be dependent on wounds, the possible activity of soil insects and the importance of wounds "that occurred incident to sprouting", as reported by Siegler and Bowman for peach (70), seems not to have been eliminated. The percentage of infection by hairy root through wounds made with a scalpel cut (28) was 71; with a bruise, 66; and with needle punctures, 41. Shallow wounds which did not penetrate to the cambium were less favorable infection courts than deeper wounds. Injuries made under ground commonly stayed open for three days but were closed after a week. The open infection court was maintained longer in moist soil than in dry soil. The wound did not need to be large. Experimentally (30, 54) tiny needles about 30 microns in diameter could open infection courts in tomato. In some cases (30) a single motile bacterium, introduced with a micromanipulator, induced infection. A high percentage of disease was secured when 100 or more bacteria were employed. Very small galls on tomato stems were induced by ". . . gently stroking the stems and petioles of tomato plants with a . . . needle previously moistened" with a suspension of bacteria (30). Usually these galls never attained a size greater than that of one or two millimeters. Just where the bacteria were introduced in these cases and why the galls never became larger have not been clarified.

Location of the bacteria in the host and their movement through the tissue are such large topics involving "secondary galls" and "tumor strands" that they are postponed until after consideration of exit and distribution.

The exit of gall bacteria (2, 10, 27, 28) from diseased tissue has seemed to be from the surface. In certain cases the bacteria have occurred not only in gelatinous material on the surface but also between the cells or in pockets that were composed of dead and disintegrating cells. With the interior growth of the galls or by other means the bacteria reached the surface from which they could be removed by water, as summarized in Table 3. The evidence (2), especially regarding cane gall, was of four types: (*a*) many cavities obviously created by bacteria near the surface held only a few or no bacteria, (*b*) bacteria were always found on the surface, (*c*) continuous escape of the bacteria has been demonstrated experimentally, and (*d*) bacteria have been observed in the process of discharge via intercellular channels.

Disintegration of galls has seemed an obvious means for releasing the bacteria. However, the large number of active secondary organisms present has made difficult the isolation of pathogenic bacteria. There was even a question whether some of the gall-formers could survive the competition.

Dissemination of the various gall organisms occurs in a variety of ways common to the dispersal of plant pathogens. Since over half of the organisms live naturally on trees and bushes, a very common means of spread apparently is with nursery stock. Several writers (*e.g.*, 28) have found that *P. tumefaciens* and *P. rhizogenes* lived in the soil for over a year, implying that if nursery plots became infected they were quite likely to remain so for some time. Although much longer periods have been recorded, the bacteria may have been reintroduced by running water or other means. Insects have been reported by various men (*e.g.*, 21, 24, 75) to carry the bacteria from tree to tree and to allow entrance through feeding or ovipositing wounds. Local dissemination by rain-washed and wind-blown droplets has been important, as in the case of *P. savastanoi* (27, 98). *P. fasciens* was seed-borne (91). Weed hosts might act as the source of infection; *e.g., Rumex acetosella* provided (51) crown-gall infection for beets. Soapwort weed was mentioned (10) as a host to *P. gypsophilae*.

LOCATIONS OF BACTERIA WITHIN THE HOSTS

Location of the bacteria in host tissue is similar for different bacteria, as might be expected from the manner in which they gain entrance through wounds. When a wound is made, the liquid from injured cells moves into the neighboring intercellular spaces and provides a direct liquid channel for the bacteria. Various details of this aspect have been worked out (2, 28, 54, 67) for crown-gall, cane-gall and hairy-root bacteria, which may apply also to other gall bacteria.

The intercellular location of crown-gall bacteria, although questioned (40, 42, 50), has been based upon the following lines of evidence: (*a*) The galls developed in the areas where liquid from wounds entered the intercellular spaces (54). (*b*) Galls developed from flooded areas when the region of the original wound was killed by heat (54). (*c*) There was a correlation between the size of the wound (30, 54), the corresponding flooding of intercellular spaces (54) and the size of the galls which developed. (*d*) The bacteria have been observed in the intercellular spaces (*e.g.*, 2, 3, 22, 32, 46, 54, 55, 67), and were often surrounded by rapidly dividing cells (54, 67). (*e*) Translocation of the bacteria and of inert material, such as carbon in suspension, has been observed through the intercellular spaces (34). (*f*) When bacteria entered a wounded cell and grew, the cell died (54). (*g*) When small numbers of bacteria were injected with a micropipette inside cells, the bacteria ordinarily died. If they survived, no division of the including cell took place (30). (*h*) The bacteria, seen between cells in living uncontaminated sections placed in agar, have been observed to grow, have been isolated and have been identified (54).

Questions regarding their locations have appeared from time to time because of difficulty in staining and in interpreting the results with a good stain. With a satisfactory section the bacteria have often been seen in an intercellular space. Sometimes they were distributed through a partly dissolved middle lamella. When such a wall was viewed from the edge, their intercellular position was clear. However, when the thin wall was viewed from the flat side, it sometimes appeared as if the bacteria were inside the cells above or below.

Various materials both inside and between the cells that might be confused with bacteria have received special attention with both crown gall and cane gall (2, 54). These materials included tannin, crystals, starch, fat globules, pectic granules, mitochondria, chondriosomes, young plastids and various other cell inclusions. Special techniques have been employed for determining the nature of each of the various items. Banfield concluded that ". . . the bacteria-like bodies observed by Smith and later by Pinoy and Nemec within tissue cells of crown galls were normal elements of the chondriome of the cells and not bacteria as they at one time believed. . . ." Five lines of evidence for this conclusion were given.

An intracellular position of crown-gall bacteria has been observed occasionally (46, 56) in wounded cells or old cells that were no longer dividing. In galls on plum the "bacteria" were observed (50) often arranged in threads inside the cells and never between the cells. However, normal cell elements may have been mistaken (2) for bacteria. If substantiated, this might be compared with the situation worked out for the legume root nodule bacteria. Intracellular positions have been observed with other gall-forming bacteria (10, 38).

As the gall cells developed for a few weeks, conspicuous swelling and multiplication of cells were found (54) about the position of the bacteria, which often indicated their location. In later stages the progression of cellular proliferation seemed like "appositional" growth (53, 79).

MOVEMENT OF BACTERIA IN TISSUE

Development of galls is influenced, doubtless, by changing bacterial locations. The manner in which bacteria move along with the liquid released by a wound and thus invade the intercellular spaces has already been discussed. Further enlargement of a gall as it involves more and more tissue has been partly explained (79) thus: as the gall develops, some of the cells are crushed, which releases the cell contents to flood still more intercellular spaces and to provide channels for further bacterial movement and activity. Under certain natural and experimental conditions, flooding of the tissues might extend for some distance. Somewhat related flooding of tissue has been of well known economic importance, for example, in water core of apples and pears, in the internal breakdown of celery, and in favoring infection of tobacco by several bacteria (35). It has been observed on many plants following rain and lowered temperatures.

Bacterial movement in artificially water-soaked tissue (54) was found throughout the 10 cm. of tomato stem that were flooded, indicating that the limit had not been reached. If the wound occurred under suitable conditions near the apex of a condensed bud, like that of a sunflower, it was possible for the liquid from injured cells to flood the intercellular spaces past a number of internodes and for the infecting bacteria to follow. Subsequent elongation of these internodes separated different portions of the infection by considerable distances. This separation was later shown by "tumor strands" and "secondary galls" (*e.g.*, 55, 67). Water-soaked areas have also been observed in other galls (*e.g.*, *Gypsophila* galls, 10). In addition to the liqiud from wounds and from cells crushed by growth of nearby tissue, the flooding of air spaces by several physiological means has been found relatively common. When flooding occurs from wounds in connection with bacteria or from vascular elements, one has perhaps the easiest explanation for further distribution of the bacteria.

Similar "secondary galls" in beets have occurred (17) after the bacteria entered the tracheids from colonies in pockets, traveled in the transpiration stream and broke out into new pockets. In hairy-root the presence of bacteria in vessels induced no changes in the surrounding tissues and was considered of no importance (28). In oleander knot "secondary galls" appeared up from the original infection and less often down from it. The travel was observed in stems (75) to be through definite channels of infection developed in the actively growing succulent tissue. These channels were often arranged in nearly straight lines and passed several internodes. "In the leaves the channels of infection apparently follow the veins but are apparently distinct from the vascular system, as cross-sections of veins and petioles fail to show the organism present in the vessels, while masses of bacteria are readily found in the parenchyma". In olive knot the bacteria moved through the vascular system to form "secondary galls" (27, 76). The bacteria accumulated in the ends of vessels (27) and seemed not to break out unless they were released into other tissues of the leaf scar when the leaf fell. With sunflowers grown in the greenhouse, "secondary galls" were found (4) developing at leaf scars.

The passage of virulent crown-gall cultures through vascular tissue has been rapid and extensive. In distances from point of entry cultures have been isolated at 8 cm. in fruit trees (86), 15 cm. in tomato (88) and 120 cm. in *Datura tátula* (86). The senior writer (unpublished) has found the bacteria in vessels of apple as far as the vessels were open. It has been suggested (88) that the accumulated bacteria in the vessels of tomato stimulated the formation of adventitious roots. The possibility that the bacteria then moved through the vessels of these roots to form "secondary tumors" (88) was suggested. With a similar idea regarding "secondary tumors", Braun said (4): "While one may not feel fully confident of the exact course followed by the bacteria. . . . It is believed probable . . . that the bacteria remain confined to the vessels and that under their influence cell-stimulating substances are formed that diffuse laterally and bring about cellular disturbances in adjacent tissues". These interesting concepts may be considered with reservation in view of three lines of evidence: (*a*) The bacteria have been present in vessels without causing galls (*e.g.*, 54) until they were released into the surrounding tissue. (*b*) The bacteria have been in contact with several kinds of uninjured living cells for long periods without causing galls (60, 65). (*c*) The formation of "secondary galls" is explained in other ways.

The results that Braun (4) secured may be clarified perhaps by the pictures of his experimental plants. These appeared long and spindly, like short-day and high-temperature sunflowers, as they developed during cold weather in the greenhouse. Such succulent plants in Wisconsin have had the air spaces of the stems flooded frequently. The internal stem pressures have sometimes been great enough even in uninoculated plants to split open the stems. Consequently inoculations made four and six inches below the growing tip might have the benefit of a continuous liquid channel for some inches both up and down. In this case the bacteria could invade the condensed bud and be carried upward still further by elongation of the subsequently developing internodes. Since Braun's photographs look like such plants, perhaps his results could be explained by various earlier reports (*e.g.*, 54, 55, 67). The question might be raised whether he always differentiated between parasitic and non-parasitic galls, discussed later, because (*a*) he secured "secondary galls" with attenuated cultures and without a primary gall, (*b*) in his illustrations these galls appeared quite small, and (*c*) he was able to isolate crown-gall bacteria from them in only a few instances. However, this last was not surprising in view of the frequent reports that the bacteria were not isolated even from primary galls. These "secondary galls" on sunflower in which the bacteria were dead, ordinarily have failed at Wisconsin to develop further unless they were taken out of the parent plant and placed on tissue culture media (31). Even in such cases the writers have experienced a high percentage of failures. Cultures of tissue, in which active growth was started by crown-gall bacteria and was continued after the bacteria were no longer present, are listed later with non-parasitic developments.

Elongated groups of proliferating cells, called "tumor strands", have frequently been found in regions between "primary" and "secondary" galls. There seems to be general agreement in the last 25 years that these strands sometimes fail to connect the two kinds of galls. Consequently the "secondary gall" does not develop as an outgrowth or branch of the primary gall. The "strands" probably grow about the narrow channels through which the bacteria pass in flooded intercellular spaces. In some cases these channels (55, 67) have been elongated as a result of expansion by condensed buds.

The distributions of bacteria by means of liquid within intercellular spaces, of elongated growing tips, and of release from injured vessels carrying bacteria permit analogies between the "secondary galls" formed by crown gall, olive knot and other galls.

The "secondary galls" on sunflower from which the bacteria could not be cultured have provided excellent material for tissue culture isolations (6, 96). When grafted back into sunflower this callus-like tissue continued to grow. Similar cultures have been secured (94) from non-parasitic galls on tobacco and other plants which are considered later.

The further extensive physiological literature about crown gall and its causal agent is here omitted because of its volume and because there is practically no comparative information about the other bacterial diseases. However, a brief mention seems appropriate of some related developments not caused by micro-organisms.

NON-PARASITIC GROWTHS

A variety of non-parasitic but pathological growths have been described which in some cases have been confused with the bacterial galls. Wound overgrowths on piece-root-grafted apple trees, for instance, were for a long time called crown galls. However, they have been differentiated from bacterial galls and their identity as a non-parasitic difficulty proved (65). They resemble the overgrowths induced by a wire girdle and by a cut made part way through the stem (63). In such cases the enlargement is associated with an accumulation of food materials as they move downward.

A closely related enlargement has frequently occurred at the union between a vigorously growing scion and a dwarfed root stock (57). Although relatively rare in the United States, it has been found frequently in Europe where dwarfed apple trees are commonly propagated.

Bur knots were considered at one time to be the result of crown-gall infection, but more recently (8, 89) they have been removed from the category of bacterial diseases. They have appeared with great frequency on seedling apple trees, and many such seedling trees have been considered undesirable because of this character. The bur knots consist primarily of clusters of root initials which appear especially near the buds on the above-ground stems. As such stems are placed in the ground, the root initials grow and permit a new apple tree to develop from this cutting. It is these bur knots which enable the Doucin, Paradise and East Malling root stocks to serve as understock for known varieties.

Non-parasitic galls have also been observed by various workers (*e.g.*, 93) on the tobacco cross *Nicotiana glauca* Grah. ♀ x *N. langsdorfii* Wein. ♂. They were covered by an abundant growth of epidermal hairs and gave rise to shoots. The disorganized tissue was primarily parenchymatous with scattered vascular elements. It contained considerable starch and tannin. When isolated aseptically with procambial strands, unlimited growth has been secured of a white callus-like growth (94). When such tissue was placed under 8 mm. of liquid, stem growing points were differentiated which developed into short stems and formed leaves. The effect was attributed to reduced oxygen supply. However, differentiation in aqueous medium was completely prevented by indole-3-acetic or naphthaleneacetic acid which led to the conclusion (71): "Under certain circumstances the oxygen gradient is an important external factor operating to prevent organ formation, but its effect must be very indirect".

Isolations and cultivation *in vitro* have also been made (reviewed, 95), for example, from roots (tomato, sunflower, radish, clover, mustard, buckwheat, pea, flax, vetch, wheat), cambium (willow, poplar, oak, beech, pine, carrot), procambium (tobacco, squash, sunflower, potato tubers, kohlrabi), and various stem tips and embryos.

Various other non-parasitic growths have been induced by chemicals (77). Among the most active have been indole-3-acetic acid, naphthaleneacetamide, and many others among the plant hormones. Galls induced by hormones have closely resembled crown gall (11, 36) and continued growth for some months. Decapitated bean plants treated with 3% indole-3-acetic acid in lanolin developed galls 2 cm. in diameter (23), mostly by growth from the pith. Irregular lateral outgrowths developed mainly from phloem derivatives. The tissues stimulated varied with different plants and different chemicals. Culture of tissue *in vitro* from galls caused by indole-oxalo-acetic acid showed (96) low growth rate and "a high degree of morphogenic conformity . . . ". Placed back in the same plant species such tissues failed to grow.

When such galls were induced by chemicals above the point of inoculation with attenuated bacteria, the tissue about this attenuated culture was stimulated as much as that about a virulent culture (5, 59). A similar phenomenon had been earlier observed when inoculations with a virulent culture were made above those with attenuated cultures (44). In some experiments (5) the inoculations and chemical treatments were so close that the bacterial or chemical cause was not too clear. The results were interpreted as follows: The "growth substances used . . . served merely to stimulate cells previously altered by the attenuated culture". When a longer distance between chemical and inoculation was employed (59), the same phenomenon was observed. Riker (59) concluded: "While one might jump to the conclusion that such a chemical is the factor missing from the region of the attenuated culture, caution is indicated because these chemicals induce considerable cell growth near the point of application. So these rapidly growing host cells may be providing the missing factor"

SUMMARY

Comparisons have been made of nine bacterial plant gall diseases, *viz.*, beet pocket rot, cane gall, crown gall, Douglas fir gall, *Gypsophila* gall, hairy root, oleander knot, olive knot and pea fasciation, as well as some similar non-parasitic galls.

Some of these galls have been known for centuries. Several have been reported from widely scattered regions where they may have been distributed with nursery stock. Their economic importance has been great in some cases and small in others.

Scientifically they are of particular interest because they provide an opportunity for studying various aspects of pathological growth. The fundamental similarity between plant and animal cells, and the relative ease with which plant cells can be studied, have suggested that a clarification of diseased growth in plants would be helpful also in an understanding of similar conditions in animals and human beings. The word "cancer" has been avoided.

Among the factors encouraging fundamental work with plants are large numbers, low cost, suitable range of types including some resistant and others susceptible, easy experimental manipulation, pathological growths easily induced by micro-organisms and by non-parasitic agencies, genetic purity through pure lines or vegetative propagation, and cultivation *in vitro* on nutrients with known chemical formulae.

Susceptible and resistant hosts and virulent and attenuated pathogens as well as host specificity have been observed.

Crown gall has appeared on many hosts; the other bacterial galls on relatively few. Investigations of crown gall and its causal agent have been extensive; those of the other diseases rather limited.

Among the bacteria many morphological and physiological similarities and differences have been listed. Production of ammonia by almost all, if not all, of these organisms is noteworthy.

A working hypothesis for the initiation of pathological growth has suggested a disturbed balance between various critical factors, including perhaps oxygen tension, nitrogen metabolism, osmotic relations, "irritating" metabolic products, food materials and growth substances.

Entrance by gall bacteria into the host is usually through wounds; their exit is apparently from the surface of living galls. Dissemination occurs in various common ways.

The stimulating bacteria inside tissues have been located between the host cells. They have sometimes been found inside cells that seemed no longer active. Although the position of the bacteria has been occasionally questioned, much evidence has accumulated in favor of an intercellular position.

Various bacteria progress through the tissue in several ways and form "secondary" galls as well as "tumor strands". For example, they move in intercellular spaces flooded by the liquid from injured cells or from physiological disturbances. In the latter case they sometimes move several inches. When the liquid containing bacteria involves a condensed bud, subsequent elongation of the internodes provides still further separation. The bacteria also travel with the open sap stream after entry through injured vessels, and form "secondary galls" when released into the surrounding tissue.

Strands of pathological tissue often extend from "primary" to "secondary galls", but there was no actual connection in a number of cases.

More or less closely related to bacterial galls are various nonparasitic galls caused, for example, by wounds, grafts, accumulated food materials, genetic characteristics and certain chemicals including "plant hormones". Callus growths *in vitro* and their grafts back into the host are noteworthy.

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ROOT DISEASES OF DECIDUOUS FRUIT TREES

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INTRODUCTION

Wherever deciduous fruit trees are grown, losses from root troubles occur. In one place one disease will be the most important and in another region an entirely different disease will prevail. The root troubles affecting the pome fruits in the main are different from those affecting stone fruits. From the orchardists' point of view, root diseases may be very disconcerting, since they usually begin taking their heavy toll of trees about the time the orchard starts bearing. Before an orchardist sets out another tree in the spot where one has died he should know what caused the death of the tree. If it was poor drainage, remedial steps may be taken. If a parasitic root disease caused the tree to die, a replant will probably die also. Even though a definite remedy for a certain disease is not known, information about the nature of the disease may be valuable.

The object of this paper is to give a brief discussion of the present status of information on root diseases of deciduous fruit trees. The emphasis is here placed on the diseases rather than on the mycological aspects of the pathogens. Such a treatment will place the emphasis on the host and the effect of various environmental factors on its resistance and susceptibility. Root diseases of certain herbaceous and woody cultivated plants have been reviewed by Simmonds (46) and by Berkeley (3), while Garrett (21) has reviewed the relation of the pathogen to the soil environment and the influence of such environmental factors on the propagation and maintenance of the pathogens of a number of root diseases. Garrett (22) has recently given a still more comprehensive treatment of the root disease problem.

The writer has attempted, as well as may be, to make this discussion worldwide in its scope and application. However, he is most familiar with conditions in the eastern part of the United States where for 12 years he worked on root diseases of fruit trees, and for that reason it may be that the root diseases of this region will be more comprehensively treated than those of other regions.

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In discussing the infectious diseases of pome and stone fruits, the more important ones will be treated first. Since many root troubles may be traced to physiological conditions, a brief discussion of the non-parasitic disorders and their possible relation to other root troubles will follow the section on parasitic diseases.

Many viruses, such as those causing phony peach, mosaic, yellows and peach rosette, may invade the roots, but since they do not produce symptoms in the root they will not be discussed in this paper; neither will root troubles resulting from improper use of chemicals for borer control be discussed.

Root troubles occurring in widely separated regions and on plants growing under very different environmental conditions have been studied by various workers who have attributed the particular types of trouble to one or another of a number of different things. Nematodes and woolly aphids often produce extensive root gall formation causing a serious devitalizing effect and therefore still further complicate the root disease problem. Many root troubles, however, can not be attributed to one single causal agent, as has often been done, but rather to a number of concomitant factors.

POME FRUITS

The principal root diseases of pome fruit trees occur on the apple, and these will be discussed first, followed by a mention of the root diseases of pear.

Apple

Black Root Rot. The most common and destructive parasitic root disease of apple trees is the black root rot (*Xylaria mali* Fromme). Most of the many species of the genus *Xylaria* are harmless saprophytes; a few are parasitic. A root disease of hibiscus (30) and root diseases of *Hevea* rubber trees (47) in the tropics have been reported as due to species of this genus. However, the black root rot (*Xylaria mali*) of the apple is the only root disease of importance occurring in the temperate zone that is known to be caused by a species of *Xylaria*. This disease is very restricted in its distribution. It occurs in the southeastern and south-central part of the United States, the region of its greatest prevalence extending from Maryland as far south as the apple is grown and west through Arkansas (10).

A tree affected with black root rot may show one or more rather definite characters which permit identification of the disease. Large finger-like fruiting bodies are frequently found at the base of diseased trees. These fruiting stromata are white at first, when an abundance of unicellular hyaline conidia are formed on them. Soon the fruiting bodies turn black, and by autumn mature ascospores have formed. A distinguishing character which is useful in the absence of fruiting bodies is the black charcoal-like stromatic coating on affected roots. The above-ground parts show secondary symptoms indicative of root trouble, but they are not distinctively different from those associated with other severe root disorders (18).

The disease spreads from root to root of an individual tree, and in three or four years a tree of bearing age may succumb to the disease. Although the disease is not highly contagious, each year a few more trees are affected so that eventually as many as 25% of the trees may have been removed from an orchard because of this disease.

Monthly inoculations over a period of several years gave an infection curve that made a rapid rise in June, reached its peak of 85% infection in July and then declined rapidly to nearly nothing in September (12). There is some evidence that this period of high infectibility in midsummer is associated with a period of low root activity of the host; that is, the curve of infection would be the inverse of the curve of root activity (12). Inoculation studies on nursery trees indicate that trees maltreated by severe summer pruning or by root pruning are more susceptible to *Xylaria* infection than are untreated checks. Field observations seem to support the hypothesis that trees growing under adverse conditions are more susceptible to infection than those growing under more nearly optimum conditions (10, 12).

Experiments are in progress to obtain more information as to whether or not replanting can be done safely where diseased trees have been removed, but the results have not been determined yet. According to the information now available it is not advisable to replant where trees that died of black root rot have been removed.

Searches for resistant stocks indicate that some stocks are more resistant than others, but a highly resistant stock has not yet been found (*39, 20,* 18, *19,* 10, 13).

White Root Rot. A white root disease of apple and other trees has been described recently as occurring in the eastern and central parts of the United States (15). The fungus causing this disease (*Corticium galactinum* (Fr.) Burt) is widespread as a saprophyte both in this country and in other countries. Since the pathogen is so widespread and attacks such a wide variety of hosts, it may be that this disease is much more extensively distributed than is now known.

The most striking symptom is a thick weft of white mycelium covering the surface of affected roots. In the initial stages of infection the mycelium gradually kills the bark from the outside inwards and gradually advances to the cambium which it kills in spots. The living tissue surrounding these killed areas may begin to lay down walling-off tissue, but before much progress has been made the fungus usually kills farther. This process leaves bird's-eye-like or zonate spots on the wood surface of affected roots. If the bark is removed these zonate spots characteristic of white root rot are visible on the surface of the wood. In some cases, before the root is finally killed, bark and wood continue to grow around a spot where the cambium has been killed, thus giving a knotty and knarled aspect to affected roots. Finally the wood of affected roots is completely rotten and therefore very soft and lightweight. The fruiting of the pathogen is an inconspicuous hymenial layer, readily distinguishable from the white mycelium by its buff to ocherous color. The hymenial layer may be formed at any time in summer or autumn when conditions are favorable, but usually in autumn. It is formed on the surface of the soil at the base of the tree or in open pockets in the soil. Small unicellular hyaline basidiospores are produced in great abundance (15).

Trees of pre-bearing age are apparently less susceptible to white root rot than those of bearing age. In the unpublished experiments of the writer there are cases in which five-year-old apple trees were successfully inoculated one year and by the next year the lesions, which evidently did not involve the cambium, were completely healed over. Field observations indicate that bearing trees are more susceptible than those of pre-bearing age, a condition that Baines (2) found to obtain with *Phytophthora* collar disease of apple trees.

The writer has made numerous observations on the distribution of white root rot and has always found it attacking trees growing in new land which has been recently cleared or in land adjacent to woodland. This root disease, unlike black root rot, affects a large number of hosts such as dogwood (*Cornus florida* L.), blackberry (*Rubus alleghaniensis* Porter), holly (*Ilex opaca* Ait.) and a number of ornamental shrubs (15). Cases are known in which ornamental shrubs were planted on stumpy land and all the shrubs near the stumps were killed by this disease. The knowledge that the pathogen is so intimately associated with roots and stumps in the soil may be useful in choosing an orchard site or in combating the disease if it gets started in a planting of ornamental shrubs.

Phytophthora Root Rot. A serious collar disease of apple trees was until recently considered to be caused by the pear blight pathogen (*Erwinia amylovora* (Burr.) Winsl.) (33). It is now known that *Phytophthora cactorum* (Leb. and Cohn) Schroet. is the cause of collar blight (2). Some varieties are much more suceptible than others, Grimes Golden being especially susceptible. Formerly it was considered that this disease affected primarily the trunk. Within the past decade, however, a form of the disease has been reported that extends well down on the roots of apple trees (57). Some symptoms of affected roots show similarity to the collar blight type of the disease; namely, a water-soaked area when the disease is active, later followed by a crack or definite line of demarcation between the healthy and affected tissue. It is possible that *Phytophthora* is more prevalent as a root rot than is now known, because the pathogen is difficult to isolate and the disease is very similar to such environmental disturbances as winter injury, and therefore diagnosis may be uncertain. Further work is necessary to determine the prevalence of and the importance of *Phytophthora* as a root rot disease of apple trees.

Crown Gall. Since crown gall (*Agrobacterium tumefaciens* (E. F. Sm. and Town.) Conn) is concerned to a considerable extent with root tissue, it should be considered in this discussion of root diseases. The workers on crown gall have recognized two types of infectious formations, *viz.*, the gall type on the collar and roots (caused by *Agrobacterium tumefaciens* (E. F. Sm. and Town.) Conn) and the hairy-root form (caused by *Agrobacterium rhizogenes* (Riker *et al.*) Conn) which is characterized by excessive fibrous root productions (38, 43). In addition to these infectious malformations caused by specific pathogens there are two non-infectious malformations, *viz.*, non-infectious hairy root (42) and non-infectious overgrowths around graft wounds. Some varieties of apples are much more susceptible to graft-wound overgrowths than others (38, 40, 45).

Crown gall may affect a large number of fruit plants, but for convenience we will discuss it among the apple diseases. It is a more serious disease on nursery stock than in orchards. As many as 50% of nursery trees are sometimes discarded because of this trouble. Infection usually takes place at the wounds made in grafting and also at wounds made by the sharp edges of the endocarp of stone fruits (44) during emergence of the seedling.

Application of control methods has reduced nurserymen's losses in recent years. Control methods include disinfecting the seeds of stone fruits (44), disinfecting seedlings (40, 45), wrapping graft wounds to prevent infection (38), and acidifying the soil (41).

Rotation of crops has been recommended as a method of combating crown gall of roses in the nursery (29) and may be useful in fruit-tree nurseries.

Rosellinia. In Europe a white root rot of grape vines and also of various fruit and forest trees has been attributed to the fungus *Rosellinia necatrix* (Hartig) Berl. which belongs to the Tuberaceae or truffle family (32). This disease has been reported recently as a cause of serious losses of apple trees in California (53). In the case of *Rosellinia* root rot, in contrast to *Armillaria* root rot, there are no rhizomorphs. Roots affected with *Rosellinia* root rot are covered by profuse cottony mycelium which extends into the adjacent soil. Very little field work has been reported on the relation of environmental conditions to infection.

A serious root rot has been reported as occurring in New Zealand, where it attacks the apple and many other woody plants. The causal organism has been provisionally given as *Rosellinia radiciperda* Mass. (17). The disease occurs only in orchards planted on newly cleared land and on land where nursery trees have been grown. In this respect it is like the *Corticium* white root rot described above (15).

Rhizoctonia. A damping-off and also a collar disease affecting layers, shoots or young seedlings is caused by *Rhizoctonia solani* Kuehn. Although this pathogen primarily attacks herbaceous plants, it may cause serious losses of young and tender layer shoots of apple trees. A method of propagating own-rooted trees consists of bending and fastening down the young trees and covering them with soil. The shoots arising from the prostrate branches are very susceptible to attack by *Rhizoctonia.* This fungus may cause damping-off about the time of emergence, or it may cause a collar blight by producing numerous deep lesions below the soil line. Control methods consisting of numerous soil treatments were tried without results (16). Possibly a change in method of propagation will need to be employed in cases where the disease is serious.

Sclerotium rolfsii Sacc. This pathogen has recently been found to cause a collar blight on apple nursery trees (8, 55, 4). This disease is not always confined to nursery trees. The author has noted orchard trees as much as four years old affected by it. In midsummer a weft of white mycelium forms on the main stem of a tree at the ground level or lower. Soon the white mycelium disappears and brown sclerotia about the size of mustard seeds form on the soil and on the trunk about the collar. Since some cover crops are so much more susceptible than others to this disease, it is not unlikely that the character of the cover crop in the rotation in the nursery or even in a young orchard may affect the destructiveness of the disease. Certain legumes, particularly *Lespedeza stipulacea* Maxim., are especially susceptible (8). Recent experiments with this disease on sugar beets show as much as 65% reduction in infection by application of nitrogenous fertilizers (28).

Phymatotrichum. The cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Dugg., may attack and kill apple trees when grown in regions where the disease abounds (49). Since the region of the cotton root-rot disease is outside the range for commercial apple culture, it does not seriously affect apple production.

Armillaria. Armillaria root rot has been reported as affecting apple trees in the western part of this country and in other countries (59, 7). In the eastern and central part of the United States it is not a serious apple root disease.

Clitocybe. A mushroom root rot (*Clitocybe tabescens* (Scop.

ex Fr.) Bres.) somewhat resembling *Armillaria* root rot has been reported on apple trees (37), but it is not known to be a serious disease on this host. A partial explanation may be that the region where the pathogen thrives is south of the region where the apple is extensively grown.

Pear

The regions in the eastern United States where pears are extensively grown are north of the region where root diseases of the apple are most prevalent. Scattered pear trees growing in home orchards near apple trees affected with black root rot have been examined frequently by the writer, but no black root rot or other parasitic root diseases were observed. Inoculation tests with *Xylaria mali* indicate that the pear is highly resistant to this root disease (10). The fact that the usual root diseases of the apple have not been reported in the literature as causing serious diseases on the pear is further evidence that the pear is probably much less susceptible to root diseases than the apple. Observations of the writer of pear trees growing in the commercial pear-growing districts of Oregon and Washington also indicate that the pear is less susceptible to environmental root disturbances than the apple.

Pear blight (*Erwinia amylovora* (Burr.) Winsl.) may occur on the roots as well as the tops and cause a serious root disease. A root form of blight infection usually is due to the spread of the pathogen downward in the trunk or sprouts and then into the roots, or rarely root infection may take place directly from the pathogen in the soil (51). The root form of this disease is confined largely to those regions of the West Coast of the United States where the temperature is warm enough for the organism to thrive. The remedial treatment for the root form of the disease involves the same method of cutting out cankers and disinfecting the wounds as is used in the control of the disease in the above-ground parts (51).

Crown gall attacks pear trees as well as other fruit trees. The discussion of this disease on the apple is also applicable to the pear.

A mushroom root rot (*Clitocybe tabescens* (Scop. ex Fr.) Bres.) has been reported as causing a root rot of pear trees (34) in Louisiana, but no other report of this pathogen's attacking the pear has been noted. *Armillaria* root rot (*Armillaria mellea* Vahl ex Fr.) attacks the pear (*7*), but it is not known to cause serious losses in the United States.

STONE FRUITS

The root disorders of the cherry are here briefly summarized separately from those of the peach and other stone fruits. They are mainly of a non-parasitic nature, and the general discussion of non-parasitic diseases applies to the cherry as well as to the apple and other tree fruits. Although the losses caused by root disturbances in the cherry are of frequent occurrence and often of great magnitude, there is very little literature on the subject.

Cherry

The cherry is very susceptible to adverse environmental conditions, especially those of the soil. This is particularly true of the sweet cherry. While the cherry is grown in a wide range of soil and climate, many of these conditions are not suited to optimum development. Perhaps because of this condition environmental root disturbances are especially prevalent on the cherry and can usually be traced to unfavorable conditions resulting from hardpan, seepage or poor drainage. *Armillaria* frequently invades roots that have been thus affected.

Peach and Other Stone Fruits

Most of the literature dealing with root rots of stone fruits is concerned with peaches. For the present discussion of root troubles the peach will be taken as representative of a group of stone fruits which includes also plums, apricots and almonds.

Armillaria Root Rot. The main parasitic root disease of stone fruits is the *Armillaria* root rot (*Armillaria mellea* Vahl ex Fr.). The fungus causing this disease is very widespread as a saprophyte, occurring on stumps in woods throughout this country and in other countries. In the western part of the United States it causes severe injury to peach and other stone fruits and occurs on a wide variety of woody plants as well as on some herbaceous ones (*27*). In other parts of this country losses are very slight from this disease (11). In certain localities, however, where there is considerable loss from root troubles, *Armillaria* root rot is present and may seem to be causing considerable loss, but other complicating factors are also operating, so that it is difficult to determine to what extent this root rot organism is a primary cause or is merely attacking already moribund roots (11). Losses from this disease are reported from South Africa and other countries (56).

An important diagnostic character is the presence of string-like rhizomorphs or black root-like growths over the surface of affected roots, hence the name "shoestring root rot". Another diagnostic character is the formation of white or cream-colored fan-shaped sheets of fungus filaments in the outer layer of the root and collar region. Clumps of tan-colored mushrooms may appear in late summer or early fall on the diseased roots or at the collar of diseased trees and serve as another identification character (27).

The extensive literature on this disease has been listed and discussed by Reitsma (35).

Probably much light would be thrown on the nature of this disease if one could correlate its distribution with the ecology of the host and of the pathogen. Although the pathogen is world-wide in its distribution, there are relatively few regions, such as semi-arid regions of the Pacific Coast of America and in South Africa and Australia, where it is reported to be a serious disease.

One of the limiting factors in the distribution of *Armillaria* root rot may be soil temperature. Inoculation experiments of peach and apricot seedlings in California showed moderate infection at 10° C. and severe infection at 17°–24° C., while the greatest root growth of the peach was at 10° C. and of the apricot at 17° C. (5). Further studies may demonstrate that the regions where *Armillaria* causes a serious root rot are regions of high soil temperatures.

From the nature of the case any remedial treatment is difficult and expensive. Thomas of the University of California (54) has successfully used a soil treatment with carbon bisulphide.

Phymatotrichum omnivorum (Shear) Dugg., the pathogen of the cotton root rot, may affect the peach and other stone fruits (49), but the region of its prevalence is not where peach trees are grown extensively.

Corticium galactinum (Fr.) Burt, causing white root rot, may affect peach and other stone fruit trees (15), but it is not now known to cause a serious orchard disease.

Agrobacterium tumefaciens (E. F. Sm. and Town.) Conn, causing crown gall, may seriously affect the roots of nursery trees by producing galls on the roots (40, 41, 44, 45). If trees having galls are culled out at planting time there is usually very little of the disease in a mature orchard. Crown gall is reported to be a serious orchard disease of almond and peach trees in certain parts of California, and in those regions it may be the limiting factor in almond production. A method of successfully killing galls on living trees has been devised (1). (See crown gall under apple root diseases.) Also preliminary investigations with penicillin in the treatment of galls on living *Bryophyllum* plants indicate further possibilities from local applications to kill the crown gall pathogen in the galls (6).

NON-PARASITIC DISEASES

It often requires considerable investigation to determine whether a certain fungus is growing as a saprophyte on a dying root or as the causative agent. Root troubles are further confused by the resemblance of a wide variety of environmental and physiological disturbances to those caused by some pathogenic organisms. Many times conditions are unfavorable for proper functioning of the host plant, and some pathogenic or saprophytic organisms growing on such weakened hosts may seem to cause trouble but in reality are largely secondary. A knowledge of these non-parasitic disturbances is requisite for a correct understanding of root troubles. The important causes of non-parasitic root diseases of fruit trees arranged roughly in the order of their importance are winter injury, poor soil aeration, drought, shallow soil, high water table and incompatibility of stock and scion.

Winter Injury. The place where root and top meet, usually spoken of as the collar, is very important from the standpoint of root diseases. This region is the last to mature in the fall, often not before cold weather (48). Root tissue is much more susceptible to cold injury than top tissue (31). Inadequate snow covering at the collar of a tree when low temperatures prevail may result in winter injury to the collar and shallow roots.

For many years collar injuries to apple trees have been known and have been the subject of investigation (23, 24, 50). This type of trouble may be observed on apple trees growing in a wide range of ecological conditions, as in the mountains of North Carolina, Virginia, Pennsylvania and New York, and in the semi-arid regions of Colorado, Utah, Washington and British Columbia. The environmental conditions in these places are widely divergent, and the complex conditions resulting in collar injury are undoubtedly very different. Accordingly the trouble has been attributed to many causes. In the irrigated and semi-arid regions it has been attributed to alkaline irrigation water, to arsenical injury (25), to excessive nitrate (26) and to winter injury (31). In the more humid regions pear blight was considered important in causing a collar disease (33).

Welsh (57), working in British Columbia, has recently shown that *Phytophthora cactorum* causes collar and root injury. This information that *Phytophthora* may cause at least one type of collar disease has thrown some light on the confusion about the cause of collar rots. However, further study is necessary to learn to what extent collar injuries are due to *Phytophthora.* Since the symptoms of *Phytophthora* and of pear blight may be quite similar to each other and since *Phytophthora* is now known to cause a disease of the below-ground parts of apple trees, perhaps some of the trouble previously attributed to pear blight and possibly other causes will be found in reality to be caused by *Phytophthora.*

Undoubtedly winter injury is one of the important factors contributing to this type of disturbance (23, 24, 50, 31). There are many factors entering into the winter injury complex other than low temperature. The physiological status of the tree is just as important as the weather. It is not possible to state a definite temperature which causes winter injury. Autumn temperatures are often so mild that trees continue to grow late in the season. A sudden drop in temperature after this mild weather may cause serious collar injury. Similarly in the spring freezing temperatures after dormancy has broken may cause serious collar injuries. In regions of low rainfall inadequate moisture at the advent of cold weather may be a contributing factor to collar winter injury.

Cold injury at the collar may be localized and thus completely kill a spot of relatively small area. On the other hand, the injury may be generalized and therefore the vigor and vitality of the whole tree be affected. Killed or weakened tissue may become a good court of entrance for parasitic or weakly parasitic fungi. Many times it is difficult to determine whether some fungus is incidental to winter injury or is the primary cause of the disturbance.

Winter injury may be intimately associated with a number of nutritional and environmental disturbances. Any devitalizing tendency may cause a tree to be more susceptible to winter injury than normally. A tree growing in waterlogged soil or weakened by overbearing is more susceptible to winter injury than it otherwise would be.

Poor aeration may be associated directly or indirectly with many types of root injury. The possibility of poor aeration should always be considered in diagnosing root disorders. Serious root trouble can often be corrected by providing adequate drainage.

Shallow soil, hardpan and high water table are related conditions which are directly or indirectly responsible for many root troubles that may be classed as drought injury.

Incompatibility of stock and scion may possibly be responsible for some obscure root disturbances where the cause of the trouble is not readily determined. Poor growth and loss of apple trees have been reported where Siberian crab was used as stock (58). Unpublished work of Guy E. Yerkes at the Plant Industry Station at Beltsville, Maryland, records a case where Stayman Winesap, Wealthy, and other varieties died within two years when worked on a certain clonal stock (Spy 227) of Northern Spy origin.

GENERAL DISCUSSION AND REMEDIAL MEASURES

Many root troubles of fruit trees are very obscure and imperfectly understood. The progress of a disease on tree roots can be learned only by digging. Since they require much time, effort and special tools, observations on roots can not conveniently be made in conjunction with other routine disease studies. Frequent observations during the year are necessary to learn the nature of a root disease; but such observations complicate the problem, since disturbance of the roots and soil to make them may create abnormal conditions.

In any study of root diseases the physiology of the host must be taken into consideration. Vigorous trees not only are less likely to develop the non-parasitic disturbances mentioned above, but they are also more resistant to parasitic diseases than are trees in unsuitable soil or suffering from improper orchard practices.

A number of remedial treatments for root rots on fruit trees as well as on other plants have been proposed, but most of them are not now in use. Some of the remedial measures that have been proposed for root rots of other hosts than fruit trees, if they had merit *per se,* could probably be adapted to fruit trees. But many of these recommendations are impractical and others are based on little or no experimental work.

Remedial treatments for root troubles are of necessity very restricted, and often they may be so slightly effective as to be impractical.

Soil disinfection will probably prove useful in certain special conditions. It is not only expensive and laborious but it has the added disadvantage when used against a disease of a perennial plant, such as a fruit tree, that if it is effective in killing a fungus parasite it will probably kill the living host plant and may even kill adjacent non-infected plants. Soil disinfection with carbon bisulphide has been successfully used in California as a means of eradicating *Armillaria* from an infested area in an orchard (54).

Fertilizing with the usual mineral fertilizer elements alone or in combination with heavy applications of stable manure was not effective in preventing infection from artificial inoculation with *Xylaria mali.* However, these tests did not include the factors of prolonged viability of natural inoculum and persistence of the disease in nature (14). Infection of *Sclerotium* on sugar beets, however, has been greatly reduced by application of nitrogenous fertilizers (28).

The field of resistant understocks has not been explored as much as it should be. Searches have been made for an apple stock that would be resistant to black root rot, and the results show that various stocks have differences in resistance, but a highly resistant one has not yet been found (20, 13, 18). Many of the possibilities in this fertile field are still unexplored. There is always the possibility that a more compatible rootstock or one better suited to some even slightly adverse soil condition will prove to be more resistant to root diseases.

Changing the soil reaction probably has very definite limitations in combating such soil organisms as *Corticium* and *Xylaria* and other pathogens that live in the roots of the host plant and thrive in the soil so long as there are roots of some host plant on which they can feed. However, the widely distributed soil organism causing crown gall does not thrive in an acid soil, and changing the soil reaction sufficiently for it to be unfavorable to the crown gall organism may be a mode of attack on crown gall in the fruit tree nursery (41).

A method of combating crown gall when it becomes established on orchard trees is the local application of a chemical to the gall. This unique type of control is applicable only to such a localized pathogen and even there it has its limitations (1).

Sanitary measures, such as hauling instead of dragging from the orchard trees that have been removed because of root disease, should be effective in preventing the spread of the pathogen of parasitic diseases such as black root rot and white root rot.

Locating the orchard on land free from stumps rather than on stumpy new land should be a useful precaution against starting an epidemic of such a disease as white root rot.

Possibly the most important application of the work that has been done on root troubles lies in the field of prevention. Most of the root troubles of fruit trees are more serious when the host plant is at a disadvantage. It is, therefore, important that the orchard be located in a region to which the particular fruit plant is adapted, and also that the site, topography and fertility of the soil be favorable so that the minimum of unfavorable environmental influences will prevail. Many of the most serious root troubles discussed in this paper are troubles of old or at least mature trees. When good culture and favorable environment are provided, a uniform stand of thrifty trees will result and profitable crops will probably be produced before these root troubles begin taking a heavy toll of trees. When such favorable conditions are provided, the orchard will probably be more profitable while it is producing and not become unprofitable because of root rot so soon as an orchard growing under less favorable conditions.

The trend of study of this group of difficult-to-control diseases will probably be more and more in the direction of learning the rôle of adverse environment in producing non-parasitic disturbances and also the effect of various environmental conditions on susceptibility and resistance to parasitic root disturbances. Probably in many cases specific recommendations for control can not be made, but an intimate knowledge of the disease in relation to all factors of environment may prove to be very important in preventing root troubles.

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PREVENTING PLANT DISEASE INTRODUCTION

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INTRODUCTION

A discussion of the prevention of foreign plant-disease introduction constitutes in reality merely a special treatment of the broader subject of disease dissemination—an attempt to view the larger field from one small angle, so as to present and interpret the conditions, relations and limitations of this larger field in terms of the human needs and peculiar interests of a given country or area. Such discussion has value only because human society has a certain confidence in its own collective intelligence and power, whereby it is encouraged to attempt a bold control of the natural world for its own benefit. Human intelligence recognizes that in spite of the universal and imposing dispersal pressure developed in all pathogens as a requisite for survival, their dissemination is everywhere checked, limited or prevented by natural means and in various ways, and recognition of these weaknesses arouses the hope, even the conviction, that with adequate knowledge and carefully directed effort a national group might be able to interfere successfully with undesirable dispersal and control its own activities so as to prevent the establishment of destructive foreign pathogens in its homeland. Stripped to bare essentials this situation represents a primal struggle between species. On the one hand are ranged a host of parasitic forms of life, numerous, successful, often advantageously specialized, always blindly surging toward new territory by virtue of a tremendous dispersal pressure, but unable to overcome certain obstacles and limitations. Matched against these is a human society, endowed with intelligence, capable of effective organization, furnished with vast means of accomplishment, but not yet fully aware of its threatened interests, and only partially awake to the character of its enemies. The struggle between these two unlike antagonists is continuously in progress and its eventual outcome lies far in the long future.

Because of this uncertain present situation it seems helpful to review the elements in the problem of plant-disease introduction from the restricted viewpoint of our own country, in the hope of bringing out certain values and relations most likely to be useful in national thinking and planning.

DISEASE INTRODUCTION INVOLVES EFFECTIVE ESTABLISHMENT

It may be emphasized that plant-disease introduction is not necessarily accomplished by mere entry into the country of inoculum in the form of spores or other living stages of the pathogen, although such entry is an indispensable first step. Introduction is not completed until the pathogen has established itself here on its host, and on an effective and permanent basis (66). It is obvious that pathogens may differ greatly in their ability to turn chance or sporadic intrusion into permanent settlement. In this respect an organism capable of attacking a variety of hosts, and able to maintain and multiply itself saprophytically, has a far greater prospect of successful establishment than an obligate parasite rigorously limited to a shuttle existence between two specific hosts. Climatic limitations, and above all those tremendous uncertainties attending the bridging of seasonal gaps, which can be overcome only by favorable adaptations or by prodigal and wasteful spore production, are often fatal to the success of an immigrant species which has been able to establish as initial foothold only a few scattered leaf spots. The chance for successful survival over winter in such a case may be extremely small. On the other hand, a pathogen brought here in a normal and intimate association with its living host is exceptionally favored and can usually survive importation difficulties almost as well as the host itself.

This emphasis on the inclusion of successful establishment as an essential element in the concept of plant-disease introduction is not so much for purposes of definition as to call attention to the wide variation that may be expected to occur in the ability of foreign pathogenic species to establish permanent residence here following slight or sporadic entry of inoculum.

REALITY OF DISEASE INTRODUCTION

Plant-disease introduction is not a mere theoretical possibility, but a tragic reality. It has already happened, and not once or twice, but over and over again in our national history. An unpublished memorandum to the Federal Horticultural Board by J. A. Stevenson in 1919[1] enumerates 120 foreign diseases known to have been introduced into this country up to that time, and the author indicates that his list is far from complete. There is no doubt that today it could be greatly extended because of later accessions to knowledge and by the inclusion of a number of diseases that have come to light more recently. Certain diseases the causal organisms of which are listed have been here since early colonial days, so far back in hazy antiquity that the date of their introduction, as well as their foreign sources, can now be nothing more than surmise. Some of the common rusts and smuts of cereals appear to have come in thus in very early times, doubtless with needed seed importations. Flax rust (*Melampsora lini*) was one of these early immigrants, as were also beet leaf spot (*Cercospora beticola*), the club root of crucifers (*Plasmodiophora brassicae*) and our common apple scab (*Venturia inaequalis*).

Later, in the nineteenth century, the vigorous growth and expansion of the country brought increased foreign trade, and with an ever-enlarging volume and variety of imports the virile young nation acquired a host of foreign pests—being heedless, we may suppose, of their evil possibilities, or perhaps it would be more correct to say, quite unconscious of their significance or even of their existence. During that century our rapidly growing agriculture took into its bosom an unusual array of troublesome and destructive plant pathogenic species, as the following items culled from Stevenson's list will serve to exemplify: Potato blackleg (*Bacillus atrosepticus*), about 1900; tomato leaf spot (*Septoria lycopersici*), end of the century; black rot of crucifers (*Bacterium campestre*), end of the century; white pine blister rust (*Cronartium ribicola*), about 1890; melon mildew (*Pseudoperonospora cubensis*), probably late in the nineteenth century; asparagus rust (*Puccinia asparagi*), early nineties; sorghum smut (*Sorosporium reilianum*), about 1880; celery late blight (*Septoria petroselini* var. *apii*), before 1890; chrysanthemum rust (*Puccinia chrysanthemi*), 1890 to 1900; onion mildew (*Peronospora schleideniana*), 1872; hollyhock rust (*Puccinia malvacearum*), before 1886; grape anthracnose (*Elsinoe ampelina*), before 1880; grape powdery mildew (*Uncinula necator*), early nineteenth century; gooseberry leaf spot (*Mycosphaerella grossulariae*),

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[1] Stevenson, J. A. Unpublished memorandum to the Federal Horticultural Board, 1919, listing plant pathogens already introduced into the United States. Courtesy Div. For. Pl. Quar., Bur. Ent. & Plant Quar., U. S. Dept. Agr.

1886; cherry leaf spot (*Coccomyces hiemalis*), about 1890; olive knot (*Pseudomonas oleae*), late nineteenth century; peach leaf curl (*Exoascus deformans*), early nineteenth century; rice smut (*Tilletia horrida*), 1890–1900; wheat stripe rust (*Puccinia glumarum*), before 1892; corn brown spot or physoderma disease (*Physoderma zeae-maydis*), probably considerably before 1900.

Since the beginning of the present century disease introduction has continued almost unabated, as the following few examples from the list will show: Citrus canker (*Pseudomonas citri*), about 1910; potato powdery scab (*Spongospora subterranea*), since 1900; potato wart (*Chrysophlyctis endobiotica*), about 1911–12; chestnut blight (*Endothia parasitica*), probably before 1900; poplar canker (*Dothichiza populea*), recent; sugar-beet nematode (*Heterodera schachtii*), recent; alfalfa rust (*Uromyces striatus*), recent; bean anthracnose (*Colletotrichum lindemuthianum*), recent; alfalfa leaf spot (*Pyrenopeziza medicaginis*), 1915; blackleg of crucifers (*Phoma lingam*), since 1900.

That this unfortunate process of introduction is still going on is well indicated by mention of a few of the outstanding diseases which have appeared since Stevenson's list was prepared. The flag smut of wheat (*Urocystis tritici*) was reported from three midwestern States in 1919 and more recently, in 1940, from Washington State. The Dutch elm disease (*Ceratostomella ulmi*) was first noted in this country in Ohio in 1931 and later appeared in several other eastern States. The bacterial ring rot of potato (*Phytomonas sepedonica*) has had a wide distribution since its recognition in 1932. The red stele disease (*Phytophthora fragariae*) was observed for the first time in American strawberry plantings in Illinois in 1935. The sigatoka disease of banana (*Cercospora musae*) reached Puerto Rico from some Central American or South American source in or prior to 1938. The golden nematode (*Heterodera rostochiensis*), a potato parasite, long known in northern Europe, suddenly cropped up in Long Island, N. Y., in 1941. Further additions to Stevenson's list were made by N. Rex Hunt in 1938, in an unpublished memorandum[2] putting on record 75 introduced diseases in addition to the 120 already listed.

[2] Hunt, N. Rex. Unpublished memorandum listing 75 introduced diseases, additional to those listed in 1919 by J. A. Stevenson, Courtesy Div. For. Pl. Quar., Bur. Ent. & Pl. Quar., U. S. Dept. Agr. 1938.

FOREIGN DISEASES COSTLY

As these examples testify, many of our serious agricultural diseases are of foreign origin (60). Each of these successful intruders, it has been constantly emphasized, imposes a heavy and permanent load on our agricultural economy, through direct crop damage, lessened yield, increased cost of production, deterioration of product, costly control measures or the resort to inferior varieties or crop substitutes. The practically complete extermination of eastern chestnut stands by the blight fungus (*Endothia parasitica*) introduced from Asia, provides a spectacular example of the destruction of a valuable national crop asset through the advent of an obscure foreign parasite (27). While this case appears extreme because of the definiteness and finality of the host's disappearance, in reality the yearly drain on national resources from many other introduced diseases may well match the impressive tribute extracted by chestnut blight.

Unfortunately, it has been found exceedingly difficult to estimate these less obvious losses with satisfactory exactness. The Plant Disease Reporter (82) has for many years published the best disease-loss estimates it can obtain from its numerous field sources of information, but it is admitted that the figures given can be, at best, approximations. The difficulties attending any attempt to express these losses in terms of common monetary values have been recognized by a number of pathologists (29, 31, 72, 73). They have also been treated in some detail in a painstaking analysis made in connection with California studies of the economic effects of quarantines (66), wherein the interaction of many diverse factors is shown to bring about a troublesome complexity in the problem of loss estimates. Yet in spite of the difficulties in loss measurement, there is little question of their reality, or even of their magnitude, as shown by such approximations as can be made.

While the vast total of the tribute exacted by various introduced diseases is thus difficult to establish, some light can be thrown on the extent of these drains on the national treasure chest through records of losses incurred or funds expended in dealing with several outstanding examples, it being understood that the figures given in each case represent only a part of the national damage bill.

The potato wart is an introduced disease of still very restricted occurrence, affecting only small, isolated areas in three States (33).

Yet for its seemingly effective quarantine and eradication measures Pennsylvania alone has expended at least $20,000 yearly for the last 25 years. The loss of potential timber caused by the white pine blister rust in young and seedling white pines cannot be accurately estimated, although on a national scale it is undoubtedly imposing. In addition to these losses, however, State and National governments (83, *512*) have provided over 40 million dollars for investigation, scouting and control of this forest enemy in the period from 1916 to 1944. Citrus canker, now practically exterminated, cost the Federal and State authorities (83, *390*), from 1915 through 1944, about 13 millions for control and eradication activities, to which must be added the value of approximately 19,500,000 citrus grove and nursery trees which had to be destroyed to suppress this introduced bacterial pathogen. Efforts to restrict the ravages of the Dutch elm disease in the eastern States have already required the sacrifice of thousands of American elms, many of them prized and valuable shade trees, and have involved expenditure by the Federal Department of Agriculture (83, *394–398*) of approximately 21 millions of dollars, to say nothing of extensive State and private expenditures. The national loss incurred through the virtual extermination of eastern chestnut stands by the chestnut blight have been estimated at over 100 millions[3].

MANY FOREIGN PATHOGENS STILL AWAIT AN OPPORTUNITY TO ENTER

In exploring the other side of the picture so as to obtain some idea of the foreign plant pathogens still awaiting a chance to enter this country recourse may be had to Stevenson's manual (74), issued in 1926, which attempts to list plant-disease organisms still absent from this country but known to occur abroad on various genera of plants. A summary has been made by the author of 50 outstanding genera in the list, selected with care so as not only to cover the general botanical range but to include in fair proportions representative hosts in the agricultural sense—cereals, grasses, legumes, roots, vegetables, fruits, forest and shade trees, ornamentals, and special crops such as sugarcane and cotton. The disease organisms recorded for all 50 genera total 1469, or an average of 29.4 for each genus. It is true that this figure is somewhat higher than the average for all the genera listed, since the chosen 50 include many well known hosts affected by numerous pathogens; further, the list doubtless involves some repetition and a few synonyms. Yet this favorable weighting is counterbalanced to a considerable extent by the incompleteness of the list when prepared, by the numerous additions that could now be made and by the reasonable assumption that if all genera had been studied as assiduously as those involving important crop plants the general average of pathogenic species per genus would be raised very materially. In any case, this sample provides a useful, if only approximate, means of estimating the general disease situation abroad, and assures us that a relatively imposing number of foreign pathogens of unknown potentialities still await a favorable opportunity to settle in our land.

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[3] Gravatt, G. F. Chestnut blight losses. Summarized from unpublished data in the Division of Forest Pathology, U. S. Dept. Agr. 1944.

Certain other specific studies support this view. A summary of foreign solanaceous pathogens, prepared by Hunt and Lohr (39) in 1943, indicates that 93 foreign species attacking the potato are still absent from the United States; a similar study by the same authors (38) lists 325 foreign pathogens attacking pome fruits (apple, pear, quince) abroad; a list, world wide in scope, of 111 fungus and bacterial parasites of rice was assembled by the Division of Mycology and Disease Survey of the Bureau of Plant Industry in 1931[4] of which number 97 were at that time not known to occur in the United States; Stevenson and Rands (75) record 417 fungi and bacteria found with sugarcane and its products, of which 98 are not known to occur in the United States including Hawaii and Puerto Rico; and Weir (86) records 291 pathogens affecting *Hevea* throughout the world.

That foreign disease organisms are continually and insistently knocking at our doors for entry is further well attested by the yearly list of intercepted plant pests published by the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture, which presents a summary of the pests found in the inspection of materials of foreign origin arriving in cargo, freight, express, parcel post, passengers' baggage, and in ships' stores and furnishings. The list for the fiscal year 1940 (79), selected as most recently representative of normal peacetime conditions, records 25,839 disease interceptions, in part referable to 325 species of fungi, bacteria and nematodes, and in part determined to genus or family only. These pathogens were found on 174 host species coming from 66 foreign countries and localities. In this sample year the constant pressure of foreign pathogens against our quarantine defense is indicated by reference to the frequency of interception of a few common examples: *Actinomyces scabies* was encountered 469 times from 37 countries; *Bacillus marginatum,* 300 times from 7; *B. vesicatorium,* 2,472 times from 4; *Ceratostomella paradoxa,* 212 times from 12; *Helminthosporium allii,* 844 times from 10; *Puccinia graminis,* 152 times from 18; *Sclerotinia gladioli,* 403 times from 3; *Uromyces phaseoli typica,* 251 times from 4; and *Venturia inaequalis,* 189 times from 32.

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[4] Unpublished list of the world's known rice diseases, up to 1931, prepared by the Div. Mycol. & Dis. Surv., the U. S. Dept. Agr. Courtesy Bur. Ent. & Pl. Quar.

It should be made clear that the figures given for these common pathogens constitute only a reasonably representative set of data and do not attempt to record every finding of the pathogens mentioned. All these plant parasitic species are already well established in this country, and a record of their frequent occurrence in foreign plant materials serves merely to emphasize the reality of the entry pressure exerted by foreign pathogens in general.

Of more direct significance in the disease introduction problem are the included notes on various important foreign pathogens encountered in inspection. Among these, *Bacterium citri* cropped up at several ports 17 times on four hosts from four foreign sources; *Cercospora musae* was determined 15 times; and lima bean scab (*Elsinoe phaseoli*) was found 304 times.

The evidence thus indicates that foreign disease organisms have been introduced many times in the past; that they have been and can be troublesome and costly; and that many more of them still await the chance to migrate here and establish themselves at the expense of our agriculture. By what means can they enter, and what are the possibilities for keeping them out?

SPECIAL RELATIONS OF CONTIGUOUS LAND AREAS

In reviewing the factors involved in such entry, it is necessary at the outset to distinguish clearly between the possibilities for disease introduction from a contiguous land area, such as Canada or Mexico, and the prospects for invasion from other continents across ocean barriers, between continuous spread (15), where because of ability to spread locally a pathogen may readily cross political land boundaries, and a discontinuous type of spread, where dissemination must surmount formidable natural barriers such as oceans. California writers (66) have pointed out the greater difficulties of excluding diseases from contiguous land areas as compared with those from overseas regions. Orton and Beattie (61) recognize fundamental differences between a foreign quarantine, which aims to exclude pests from abroad, and the domestic type of quarantine, employed to prevent, or minimize, or delay the spread of some insect or disease within continuous land areas, the latter having far less chance of permanent success than the effort advantaged by natural barriers.

The risk of introducing diseases from adjacent countries thus merits special attention. Land continuity not only connotes identity of crops and agricultural practices on both sides of the common border but encourages an intimate and extensive exchange of products. Along with these favorable conditions for disease transmission there must be associated the hopeless prospect of trying to prevent an actively spreading pest from crossing a purely imaginary line, either through the air itself or by way of a host of articles and materials impossible to bring under effective quarantine control.

Yet the apparent futility of protective measures at such a land boundary loses something of its significance when one considers that under stable agricultural conditions most pathogens indigenous on the natural flora of the adjoining country may be practically ignored. If they have not been able to reach our territory in ages past, it is likely that they are subject to climatic or other limitations and need not be feared. This viewpoint toward indigenous pathogens holds likewise for many long-established pests on staple crops in the adjoining country. In the 300 years previous to initiation of the last 30 years of quarantine effort there have been ample opportunities for entry into this country from Canada and Mexico of their long-present crop diseases, indigenous and acquired. If these have not yet crossed our borders, even where special crop-bridging conditions have become established, it is almost necessary to assume natural limitations on spread, limitations presumably as effective today as in centuries past.

In the light of this situation the threat of disease introduction from our neighboring land areas is markedly lessened. But it is not eliminated. There still remains a danger—in new pests introduced from abroad into the neighbor territory; in new crops which may alter long-established pest relationships; in new crop areas which may constitute a bridge to dissemination hitherto lacking; and in the commercial development of new products the importation of which may provide an effective channel for the entry of previously well isolated pests. In all these cases it will be apparent that the disease exclusion problem is almost identical in its biological features with that encountered in attempting to prevent spread of a pathogen from one area of our own country to another.

Because of the difficulties involved in preventing disease spread to us through a continuous land area, it is recognized that the introduction of a destructive pathogen into a neighbor country constitutes almost as great a menace to us as would its advent into our own land. To meet this situation it has been urged (28) that the several countries lying within the protection of effective natural barriers could well unite in a general scheme for the exclusion of foreign pests from their common territory.

NATURAL CHANNELS FOR THE INTRODUCTION OF PLANT PATHOGENS

Disease introduction from overseas regions is subject to much different conditions than that from contiguous land areas, both as to means and facility of entry and as to natural barriers and transport limitations. It is important to understand well the several channels through which foreign plant disease organisms may reach this country from overseas, including the avenues provided by nature as well as those created by human activities. The chief natural channels of disease spread comprise air currents, surface water flow, insect activities and animal movements, particularly the flight of migratory birds. In the field of human affairs various phases of war, migration, commerce and travel are concerned. While the natural means of pathogen introduction are largely beyond human control, it is still very necessary to gauge their effectiveness and particularly to determine their limitations, in order to assure that ambitious exclusion efforts in the field of human activities will not be weakened or nullified by the entry of foreign pathogens through these natural paths.

Land-water flows. Run-off, floods, rivers, streams and irrigation waters are undoubtedly effective in the local distribution of some pathogens (23). Yet, while these could have some significance in the movement of pathogens between limited areas of this country and adjacent portions of Mexico and Canada, in the field of intercontinental spread of present interest to us they would play no part.

Migrating birds. In their migrations birds could be suspected of carrying pathogens both externally and in their digestive tracts. Yet there appears to be no definite evidence (66) of disease introduction traceable to this source. In fact, after much study of the plant-quarantine relations of spore dissemination, Butler (14) concludes that bird migration plays little part in long-distance spread of plant pathogens.

Wild animals. These rarely range far enough from their customary haunts to constitute an appreciable factor in local disease distribution, and of course need not be considered in intercontinental spread.

Insects. Although normally closely confined to a given habitat, insects may at times migrate or be carried by wind in considerable numbers to distant areas (24) and could thus transport incidentally acquired pathogens. While Gardner (23) has dwelt at some length on the rôle of insects in spreading diseases locally, there seems to be no direct evidence that foreign pathogens have been thus introduced, and the comparative rarity of insect flight from abroad across ocean or mountain barriers appears to indicate a relatively small danger in this channel of disease entry.

Air-borne pathogens. It has long been recognized that bacteria and fungus spores are readily transported in air currents for considerable distances. Because of the pronounced change in surface and volume relations at the size level of fungus spores (13, 16), these spores, as well as bacteria and dust particles, tend to fall very slowly in air and are thus easily carried aloft in light convection currents or may be borne horizontally over wide areas by even gentle winds. The possibility of disease introduction from other countries or continents through the agency of wind-borne spores deserves careful consideration, therefore, since this channel is entirely beyond human control.

Calculations based on observed rates of spore fall in still air (48) indicate that an average oval spore type measuring 20 by 12 microns is likely to fall at the rate of about 6 mm. per second, or 72 feet per hour, or one mile in three days. Theoretically a 20-mile breeze during this 3-day falling period could carry the spore 1,440 miles horizontally before it finally reached the ground. It is clear, therefore, that the feeble tendency to fall presents scarcely any obstacle to long-distance spore dissemination.

The ready ascent of spores to upper air levels is likewise easily accounted for if one notes how much a light convection current rising at the moderate rate of one mile per hour overmatches the falling speed of the spore example cited, which is 72 feet per hour. It is reasonable to suppose that spores can be carried upward as far as these thermally induced convection movements reach. Data on the actual spore population of the air, obtained by cultures, smeared glass plates and various trap methods, indicate that under favorable conditions the air may contain surprisingly large numbers of spores. Considerable information on the nature, number and distribution of air-borne organisms, including plant pathogens, together with numerous references to the literature on air-borne fungi and bacteria, has been summarized by various authors in "Aerobiology" (56). One collaborator, Christensen (16), reports a series of 18 glass-slide spore-trap counts for wheat stem rust (*Puccinia graminis*), one of which reached a maximum deposit rate of 181,200 spores per square foot, with an average for all of 36,452, a rate of 253 per square inch. There is likewise cited by Keitt (40) Heald's determinations on the air population of spores of wheat bunt (*Tilletia tritici* and *T. levis*), which at a distance of one and a half miles from threshing operations gave a weekly fall of the order of two million spores per square foot. These records tell us further that the spore population of the air is denser near the earth's surface, rapidly lessening at altitudes above about 8,000 feet, although spores have been recovered at a height of 26,000 feet, and, in one instance, at 36,000 feet.

The evidence for actual disease dissemination locally by way of the air has been discussed by Keitt (40), and scarcely needs further comment, since both its reality and its effectiveness have long been universally accepted. For longer distances approaching those involved in transoceanic spread, the case is not so clear. Yet the evidence for effective spread of wind-borne spores over considerable distances, while circumstantial, is convincing enough in a number of outstanding cases. Among these the black stem rust of wheat stands as a classic example, not only because of the amount of attention devoted to it and the results achieved, but because it has been possible to check these results through several different lines of attack. The summary presented in "Aerobiology" by Christensen (16) indicates not only that spores of wheat stem rust are carried northward in wind from wheat fields in Texas to the northern grain lands of the United States and Canada, but that a reverse movement takes place later by which inoculum from northern areas again infects young Texas wheat, this two-way movement covering a north-south sweep of more than 2,000 miles. Stakman (68) presents evidence that spores of this rust may have traveled by air 1,000 miles in two days.

Craigie (18) in Canada observed stem rust infections 200 miles away from wheat lands at Fort Norway, 500 miles from any source, and at Fort Churchill, 700 to 800 miles beyond wheat culture in the Peace River Valley, and concluded that "there is undoubted evidence of the dispersion of stem rust spores over distances of several hundred miles".

The white pine blister rust (*Cronartium ribicola*) also supplies its quota of evidence. Pennington (62) found *Ribes* infection 110 miles from the nearest pine source of infection; and Christensen (16) cites evidence obtained by Lachmund that aeciospores of this rust may be carried 300 to more than 400 miles by the wind in the Pacific coast region.

While the implications of the evidence outlined would seem to support the view that intercontinental disease spread is at least an easy possibility, certain obstacles to such long-distance transmission have to be considered (49). The earth's air covering forms a rather thin, superficial layer, and the denser surface portion, in which spore movement largely occurs, is only five to seven miles in height—roughly, the stratum in which are concentrated most convection movements and weather phenomena. When one attempts to picture horizontal spore movement on a global scale in this turbulent and uncertain lower atmospheric layer, it will be apparent that the intercontinental spore traveler must move practically hugging the earth's surface, threading a long path through all the ebullitions of this thermally disturbed contact portion of the gaseous envelope. To the vagaries of countless local rising and falling convection streams there must be added the possibility of being caught and brought to earth in condensation droplets. Sunlight and desiccation are sometimes fatal to spores, and winds may fail or change their direction. Many fungi and practically all bacteria, nemas and viruses are ill adapted to wind dissemination, and of those fungi normally spread by wind comparatively few would be able to produce a spore population numerous enough to overcome the extremely small chances of eventual success involved in a long and hazardous overseas journey. We may therefore conclude that while transoceanic spread is undoubtedly a possibility it is at best subject to terrific adverse odds. And it may be added that the present limited distribution of the great majority of pathogens after long ages of opportunity for intercontinental dissemination strengthens the belief that such spread must be an extremely rare occurrence in nature.

INTRODUCTION THROUGH HUMAN ACTIVITIES

War and migration. Most writers agree (14, 61, 66) that the activities of man himself provide by far the most effective means for disease introduction. In war the movements of troops, with their food, baggage, supplies and plunder, can readily introduce pests to new areas, and the stresses and exigencies of the moment are usually great enough to outweigh consideration of the permanent evils which new crop pests might saddle on the home country. Sasscer (64) brings convincingly to attention the irregularities and disturbances which the present war has created in the customary inflow of plant materials from overseas regions, not only in type but in volume and manner of transport; and it has been suspected (77) that the flag smut of wheat (*Urocystis tritici* Koern.) may have been introduced into this country through importations of Australian wheat during the first world war. The disturbed and largely uncontrolled conditions attending mass human migrations can also be expected to spread crop diseases, and there is no doubt that the rapid settlement of the American continent was accomplished by frequent disease introductions, just as it brought many European insects and weeds.

Plant materials for propagation. Under more stable conditions, however, the most important channel for disease introduction lies in orderly human travel and commerce, and particularly in foreign importations. Imported shipments of plants and plant products are accompanied by living stages of foreign pathogens, not rarely or occasionally, but frequently, almost invariably. Some distinction is usually made, however, in the disease-carrying proclivities of various types of plant products. By almost universal consent plant materials for propagation purposes are accorded first rank in the culprit list (61, 66). C. L. Marlatt, chairman of the Federal Horticultural Board, in explaining the necessity for restrictions on nursery stock, stated in 1923 (44, *18–26*) that "imported nursery stock and other plants and seeds have been the source of the introduction of some 90 per cent of the insect pests and plant diseases which have come to us from other countries, and which now occasion losses to our agriculture and forestry of approximately one billion dollars annually". It has been pointed out repeatedly that an imported plant normally carries with it many of its local parasites; that these may naturally survive and continue to flourish in the new habitat as in the old; that the very care and attention given to the incoming plant to insure safety in transit and growth vigor on arrival at the same time provide favorable conditions for accompanying pathogens; and that a more certain method for deliberately transplanting a pathogen could scarcely be devised than that of transporting and planting the affected host.

Among the various classes of propagating materials, rooted plants of woody type have long had an unsavory reputation as disease carriers. Representing usually several years of growth, they have had ample time in which to acquire a full quota of parasites, and leaves, bark, buds, foliage and root system afford excellent harborage for a variety of pests of external character, to say nothing of those borne internally. The pest-carrying possibilities of woody plants are so well recognized that it has been a long-established plant quarantine policy to discourage the importation of the complete host plant wherever possible, or to limit importation to the youngest practical stage of plant development. Seedlings and rooted cuttings of herbaceous type are looked upon with somewhat less distrust largely because of the shortened time element, while woody cuttings, buds and scions stir still less apprehension, being of small size, devoid of roots and usually amenable to treatment.

Bulbs, tubers, corms and rootstocks may not carry readily the more typical stem and leaf diseases except in an incidental way, but they are almost ideal agents for spreading systemic diseases and a number of soil troubles. At least 20 pathogens can be carried by potato tubers; the sweetpotato is credited with 17; *Allium* with 19; and iris with 14.

The rôle of seeds as disease carriers is almost notorious, for the number of diseases carried by them is imposing. From the list of seed-borne diseases compiled by Orton (58), and admittedly far from complete, we gather that the seeds of 26 cereals and grasses may carry 186 pathogens; 25 vegetable seeds could transmit 115 diseases; those of 23 field crops, 100; 29 flower seeds, 40; 14 trees and shrubs, 20; and 14 wild plants mentioned are capable of spreading 16 diseases on their seeds. Cereal seeds stand out prominently as carriers of disease organisms. Wheat seeds bear 55; rye, 25; seed corn, 26; oats and barley, each 14; sorghum, 9; rice, 11; and five may occur on millet. Among other field crops, soybean heads the list with 16 seed-borne pathogens; cotton has 15; red clover, 14; flax, 9; tobacco, 6; and alfalfa and cowpea, 5 each. Some vegetable and garden seeds deserve mention. Beans may carry 17 diseases; tomato, 14; lima bean, 13; peas, 11; peppers, 8; and cucumber and beet, 6 each. Of the 477 pathogens reported by Orton as present on 131 kinds of seeds, 115 are bacterial; those of fungus diseases number 334, including 8 rusts and 30 smuts; 12 are due to nematodes; and in 15 cases virus diseases could be carried in the seed. Thirty seeds out of the 131 listed are known to carry 55 diseases not present in this country.

Plant materials for manufacture and consumption. Although importations of plant products, such as fruits and vegetables and similar materials intended for consumption, processing or manufacture, are doubtless accompanied by a wide range of pathogens affecting their respective hosts, such importations are in general considered to be somewhat less dangerous from the standpoint of disease introduction than propagating materials (66). Many of these fruit and vegetable products come from climatic zones containing few parasites dangerous to our plant species. Certain materials arrive in processed or semi-processed condition, already largely divested of their parasites. Others are destined here to heat or chemical treatment or to processes of manufacture generally effective in destroying or locking up incidentally borne pathogens. In fresh fruit and vegetable shipments we can take into account that cultural practices, commercial standards and trade demands encourage the elimination at source of a high proportion of obviously diseased units, and that cooking, garbage-disposal methods and scarcity of hosts in centers of urban consumption tend to lessen materially the chances of pathogen transfer to domestic host plants. Certainly the would-be immigrant parasite is subject to far greater handicaps when brought here on consumption products than when entering on materials designed for propagation. Nevertheless, these impediments to successful establishment constitute difficulties merely and not impassable barriers. Nor are all disease organisms entering on imported fruits and vegetables hindered alike by these difficulties. To some the situation presents an almost insurmountable obstacle to successful establishment, while in other cases the march of the parasite from imported material to local hosts is scarcely impeded. It may be concluded, therefore, that these various materials imported for manufacture or consumption constitute an important medium through which foreign pathogens may be introduced (47), in some cases with difficulty, but in general, when their great volume is considered, effectively.

Incidental entry on non-host materials. The constant disturbance of air spreads minute organisms far and wide locally; insects, birds and other animals distribute spores and bacteria freely in their movements; and human activities likewise smear or scatter spores over many articles and objects handled by workers. Pathogenic organisms are, therefore, plentifully dispersed over a considerable range of non-host materials, and if these are imported they undoubtedly bring with them much of the spore load incidentally acquired. Rye straw might thus contain spores of wheat smut, and tomatoes could be contaminated with citrus canker bacteria. In fact, imagination need not run wild to picture wheat rust in trousers cuffs or clover mildew in a felt hat. Countless articles of this non-host type arrive every month in baggage, cargo, packing, mail and personal belongings from foreign lands. What is the danger of disease introduction from the spore load such materials undoubtedly carry?

There seems to be little satisfactory evidence on this point, and an estimate of its importance must be made almost solely on general biological features. While these incidental carriers loom large in number and variety, the great majority usually arrive in small and isolated units which are widely dispersed. The scanty spore load originally acquired by some chance contact is subject to depletion by losses along the way, and by a usually high rate of mortality; chances for transfer to a suitable host are very small and, for such as go into drawer, shelf or storage, practically infinitesimal. It is possible, of course, that a seed necklace now and then will escape its usual bonfire fate and end in a bean field, or that a rice-straw doll will reach growing grain instead of yellowing into harmlessness over the years on some mantel. But even then successful infection by the scanty pathogen remnant presents enormous difficulties; the odds are still heavily against a successful establishment. It is possible of course that fortune might be favorable all through the irregular series of events, but ordinarily the chances seem to be decidedly unfavorable to disease introduction by such means. In any case it is a risk that must be accepted, since nothing short of extreme national isolation would effectually close this channel of disease entry.

Soil. As a carrier of plant diseases soil merits special attention. It is an incidental catch-all and repository for bacteria and wind-borne spores, and for those brought down in rain or snow, or spattered from plants, or acquired from fallen leaves and fruit. But it may also harbor pathogens in saprophytic form, or encourage the development of perfect stages, or provide a normal home for soil-inhabiting organisms. It is true that bog peat, subsoils, mineral earths and ocean sands are relatively barren and far less likely to carry pathogens than cultivated soils; on the other hand, miscellaneous lots of field earth, garden loam, vegetable mold and forest litter all may be expected to transport with them a more or less complete and faithful representation of the. fungus flora of their neighborhood. Plant quarantine authorities regard such soil with natural distrust and are exceedingly loath to have it imported. United States plant quarantine regulations specify that the roots of all plants from overseas shall be free from soil (*78, 21–22*). Root, tuber and bulb fungi, such as those of potato wart, club root of crucifers, and many others, infect their hosts naturally from soil and are normally at home in this medium, as are crown-gall bacteria and many nematodes. On the other hand, certain bacteria such as those of pear blight (*Bacillus amylovorus*) and cucurbit wilt (*B. tracheiphilus*) and many aerial plant parasites such as bean anthracnose (*Colletotrichum lindemuthianum*) and loose smut of wheat (*Ustilago tritici*) (33) find soil unfavorable for any lengthy sojourn, dying out in comparatively short periods.

RELATION OF PATHOGEN GROUPS TO ENTRY CHANNELS

In estimating the danger in these several channels of introduction, account has to be taken of the varying abilities of the various types of pathogens to make use of them.

Bacteria. Being poorly adapted for wind dissemination, bacterial organisms have little chance for intercontinental spread in air currents (23, 16). As many pathogenic bacteria are adversely affected by desiccation or lengthy exposure to light, dispersal by inert carriers is not always an easy matter. The same limitations materially lessen the chances for successful establishment when bacterial inoculum does reach our shores. Bacterial pathogens must largely rely for introduction on being carried with a host or its products.

Fungi. Fungus pathogens are in better case. As already noted, intercontinental air transport is a definite possibility, at least for those species producing a prodigious spore population adapted to wind dissemination, although such distant travel is always made against such heavy odds that it has but little hope of success. Fungus spores, in general, do not succumb as readily as pathogenic bacteria to drying-out or to light, and their chances for successful establishment on arrival are, on the whole, considerably better. This is especially true when the immigrant species can live and multiply saprophytically or establish a more or less permanent relation with its host, or can produce a quick succession of generations on a chance host, or is specially adapted to bridge unfavorable seasonal gaps.

Virus diseases. Although little is known about air transport of virus diseases, observation would indicate that there appears to be small danger of virus disease introduction by way of air, since there is practically no evidence of their local spread by wind. Likewise, for the most part incidental carriers do not spread viruses readily; the ease with which tobacco mosaic is transmitted in inert materials, mostly tobacco products, is somewhat exceptional in this field (33). Even soil that safely harbors many fungus and bacterial pathogens is generally a poor carrier for viruses, which are largely carried overseas within affected hosts.

Plant parasitic nematodes. Nematodes make practically no use of the air, even for local distribution, and thus need not be expected to come from abroad in wind. They can take advantage of ordinary incidental carriers to only a limited extent, but rely heavily on the soil or on the host or its products for dissemination.

When the danger of disease introduction is thus examined from the point of view of the capabilities and limitations which these pathogen groups exhibit in taking advantage of various channels of entry, it is apparent that for all of them the importation of the infected host itself offers the most favorable means of entry, closely followed in importance by host products. Soil comes next as a useful carrier, with other incidental materials close behind and air currents occupying the least important place in the danger series.

RELATION OF BIOLOGICAL RACES TO DISEASE INTRODUCTION

In the early days of biological study, the prevailing concept of a natural species envisioned a homogeneous group of individuals, together constituting a stable entity capable of modification only through the slow and extremely uncertain process of evolution. It was recognized, nevertheless, that breeding and selection of domestic plants and animals, in progress throughout human history, have produced plant and animal types remarkably different from their wild progenitors. But it was supposed that this plasticity of species was present in only a few natural groups, the vast majority of species maintaining a stolid and largely unalterable stability. Ambitious and frequently successful efforts to produce crop-plant varieties resistant to specific diseases have aimed to induce changes or combinations in a genetically plastic host while assuming a rigid inertia in the parasite. But more recent advances in knowledge tell us with increasing insistence that many pathogens are likewise plastic; they are endowed with a disconcerting variability which enables them to originate new races and strains, presumably fitting the species successfully to parallel transformations in the host with their own blind but at times effective adaptations. Rapidly accumulating evidence indicates that species plasticity is highly frequent among fungi, as a few examples will illustrate. One hundred and eighty-nine races are now recognized for the wheat stem rust (*Puccinia graminis tritici*) (1, 22), No. 189 being an exceedingly virulent type found as yet only in Peru but known to be capable of causing heavy infection on the wheat varieties used in race identification. A realistic picture of the disturbance which such biotic forms may introduce into the effort to breed rust-resistant wheat varieties is provided by the painstakingly accumulated data on the occurrence and changeful behavior of the numerous domestic stem rust races assembled by a group of workers in this field (70).

Yet this race multiplicity in stem rust, striking as it may seem, is by no means unique among fungi. The genus *Fusarium* is equally notorious for the race versatility of many of its species, as shown both in culture sports and in the varied relation of numerous races to their hosts. According to Wellman and Blaisdell (87), certain variable cultures of *F. bulbigenum lycopersici* may run a whole gamut of different strains in successive generations. It has been demonstrated (69) that corn smut (*Ustilago zeae*) can produce divergent strains freely, 162 having arisen from a single original cell, and over 1,000 from two mated germ cells. A similar species variability exists in pear scab (*Venturia pirina*) (41).

Alexander (2) reports a new strain of *Cladosporium fulvum* on tomato in addition to the four already known, and suspects that this newcomer may have been widely dispersed on tomato seed. Holton and Johnson (35) conclude that the two flag smut infection centers in the United States represent two distinct strains of *Urocystis tritici* on wheat, while Yu *et al.* (89) recognize five strains of this smut species in China. Bunt on wheat (*Tilletia tritici*) comprises 14 races, and its relative, *T. levis,* 10 (36).

Five strains of *Cercospora oryzae* are reported on rice (63), and Braun (12) found the potato wart pathogen, *Chrysophlyctis endobiotica,* to contain three races differing considerably in their behavior toward hitherto resistant potato varieties. Heald (33) notes the occurrence of numerous biological races in connection with black knot (*Plowwrightia morbosa*), corn smut (*Ustilago zeae*), ergot (*Claviceps purpurea*), powdery mildews (*Erysiphe* and other genera), white rust of crucifers (*Albugo candida*) and *Rhizoctonia solani.* These few examples could be expanded into a very extensive list.

The occurrence of different racial strains in bacterial pathogens is exemplified in common scab of potato (*Actinomyces scabies*) (76), and nematodes provide many examples of race variability (25, 71). In virus diseases, likewise, behavior patterns suggest the existence of differences in the causal agent which must be accorded racial rank (5, 20, 34).

Recognition of this widespread occurrence of biotypes among pathogens has a far-reaching significance in the problem of preventing plant-disease introduction. If we are compelled to regard each parasitic species not as a simple entity but as an assemblage of races and strains and biologic forms differing materially among themselves in host relations and degrees of virulence, then the already numerous potential pest species abroad are, for all practical purposes, multiplied many times over. This altered viewpoint affects the domestic situation as well. The earlier attitude was that, once a foreign pathogenic species became firmly established here and was widely distributed, it ceased to be a matter of foreign quarantine concern. The Plant Quarantine Act of 1912 (84) inferentially adopted this viewpoint by providing in Section 7 the power to embargo against specified foreign plant materials on account of insects or plant diseases "new to or not theretofore widely prevalent or distributed within and throughout the United States." This attitude will doubtless undergo some modification to accord with the biotype concept, since, as Stakman has pointed out[5], the introduction of a more virulent foreign strain of one of our long established domestic pathogens might have as much economic significance as the entry of an entirely new parasitic species. In other words, a national foreign disease-exclusion program should logically include consideration of foreign races and strains of our long-familiar pathogens as well as the equally variable and complex species not yet introduced here.

This matter of biotypes carries still further implications. If pathogenic species both at home and abroad are constantly, or at least frequently, developing new biologic forms, a hitherto confident reliance on our ability to breed permanently resistant varieties is materially weakened. At least future efforts to produce disease-resistant host varieties must take well into account the complex and variable nature of the pathogens concerned (69). And it is likewise apparent that biotype prevalence brings a discouraging prospect to advocates of the "open door" policy, who suggest allowing free entry to all foreign pathogens with the hope of attaining a final host-parasite stability when we have at last acquired the globe's assorted pests. If pathogens throughout the world are capable of generating a continuous succession of new races and strains, the troublesome stabilization process can no longer be envisaged as a short if hectic period of adjustment, but promises to stretch on and on into remote infinity.

NATURAL BARRIERS TO DISEASE INTRODUCTION

Studies of plant pathogenic species in various parts of the world assure us that in spite of their abundance and variety the majority of pathogens have still but limited distribution. Each has successfully survived throughout the ages, has matched the evolutionary modifications in its host with its own special adaptations, and has developed prolific means of dispersal and infection to guarantee its continued maintenance. Yet comparatively few have attained world-wide distribution; most of them are still found only in a valley, an island, a region or a continent. This localized occurrence is not an immutable limitation, however, since experience has demonstrated over and over again that many species of these parasites are able to flourish in new areas and on new hosts when artificially transplanted. Distribution evidence thus indicates beyond a doubt that most pathogens are prevented from occupying all their possible habitat by natural barriers. That these barriers are constant and effective is well attested by existing species isolation, an isolation which must have continued unbroken throughout the long evolutionary process of species differentiation. Thus there appears to be strong reason to rely confidently on the faithful protection afforded by natural barriers, and in national planning to take full advantage of the obstacles they set up to plant disease introduction.

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[5] Stakman, E. C. Unpublished address, plant pathology seminar, Beltsville, Md., Oct., 1943.

Large bodies of water. Bodies of water of large size, particularly oceans, are thought to be effective barriers against intercontinental spread of plant-disease organisms. Butler (14), emphasizing their importance, reviewed the world spread of several outstanding diseases and concluded that "in none that I have been able to find is there the slightest evidence that the spores can cross the ocean borne by the wind". Massee (46) reports about 30 species of *Puccinia* on wild plants in Scandinavia and nearby parts of Europe but not found on the same hosts in Britain, while an equal number of British species are absent from the wild plants of these countries. Meier (52) reports spores caught from an airplane over the Caribbean Sea 500 to 700 miles from land, but Bisby (8) found the air in the mid-Atlantic practically free from organisms, and the "spore-traps" operated by Lindbergh in an arctic trip (53) obtained few organisms over the ocean between Greenland and Europe. Likewise, Durham (21, *48–54*) collected few spores in the ocean air in several airplane trips made between New York and Bermuda. Orton and Beattie (61) consider natural barriers as chiefly continental, thus emphasizing the rôle of oceans in protecting this country.

Mountain barriers. High mountain ranges also interfere seriously with the dissemination of plant pathogens, partly by limiting the host range and partly because of the physical difficulties encountered in air transportation. The low temperatures at high altitudes, though not often directly destructive (17, 54), tend to involve the drifting spores in water condensation, thus bringing them to earth in rain, snow or mist. The protective effect of mountains has been noted in California (66) and elsewhere (14, 22, 57, 61). Wide areas of desert country present a formidable obstacle to spread, and zonal and climatic limitations on host occurrence create a situation unfavorable to pathogen distribution, especially of those species ill adapted to long-distance air transport.

Climate. Climate itself may restrict spread in numerous cases. Potato wart (7) and powdery scab (33) are among the diseases subject to definite climatic limitations, being practically confined to areas with cold soil conditions; obversely, the heat-loving southern blight (*Sclerotium rolfsii*) and the koleroga disease (*Pellicularia koleroga*), both attacking a wide range of hosts, seem unable to persist in northern temperate regions. California plant quarantine writers (66) mention diseases unlikely to be established in that State on account of unfavorable climatic factors. Butler (14) has noted that many northern European pests are absent from southern areas of Europe and *vice versa,* although the way is open for spread in both directions. Gratz (26) states that *Verticillium* wilt of potato is severe in Maine in certain seasons and negligible in Florida, while bacterial blight is prevalent in Florida and unknown in Maine.

FACTORS AFFECTING NATIONAL EFFORTS TO PREVENT DISEASE INTRODUCTION

General policy. When a nation becomes sufficiently aware that its vital interests are threatened by foreign pest introduction, and explores the possibilities of adequate protection, it discovers very soon that exclusion measures which would seem to be required from the biological point of view can not always be put into effect. Even when the national attitude rises far above an "open door" policy and looks with earnestness toward effective exclusion of alien plant enemies, the hoped-for degree of protection is rarely attainable. That goal is seen to involve large-scale action of an extreme isolationist character, a course often in conflict with important national interests and quite beyond what national discipline will endure. The result is compromise. Generally speaking, the only realistic policy possible may be thus formulated: In accordance with powers granted, and with the knowledge and facilities available, the national authority aims to prevent the introduction of foreign pests injurious to all branches of agriculture to the extent that public interest will permit and public opinion support (50).

Any exclusion attempt is thus saddled at the outset with handicaps, either unavoidable or reluctantly incurred because of the compelling necessity of compromise. The same need for compromise permeates throughout all phases of protective planning and procedure; everywhere the ideal course of action has to be modified for the sake of economy or other reasons, with the inevitable introduction of risks. Since these risks are not only ever present but inescapable, the quarantine planner can only study them with care in the hope of finding ways to compensate for or minimize them. Risk elements inherent in the several channels of entry, in the characters of the pathogens themselves, in cultural development and in national policy have been touched upon elsewhere. In addition to these, several other aspects of risk deserve to be estimated as accurately as possible.

Uncertainty in behavior of foreign pathogens here. A troublesome factor of uncertainty, and therefore of risk, lies in inability to forecast the probable behavior of foreign parasitic species when introduced here (14, 61, 66). Lengthy experience has provided enough striking examples to convince us that a pathogen regarded abroad as of trivial or minor character may become a formidable and destructive crop enemy when established here. Chestnut blight, citrus canker, walnut blight and white pine blister rust provide outstanding instances. Doubtless the reverse situation occurs at times, and threatening foreign pests have not here lived up to their evil reputation abroad; but this thought brings scant comfort to the national planner who still remains unable to predict confidently how any given pest would behave under our conditions. Lack of knowledge of susceptibility and resistance factors in domestic host species and varieties in relation to an untested foreign pathogen appears to be an outstanding element in this uncertainty. Since it is obviously dangerous, or at least undesirable, to bring here for testing purposes a pathogen of unknown propensities, it has been proposed (59) that, whenever possible, resistance or susceptibility be determined safely in advance by sending American plant selections abroad where they can be grown exposed to the disease in its homeland. Yet this procedure, although helpful, would not completely eliminate the uncertainty, since soil, climate, cultural practices and control measures may also modify at times the severity of an introduced disease.

Plant and climatic zones involve variable introduction risks. Importations from different plant or climatic zones of the world present a varying amount of risk (14). Both parasites and hosts tend to be confined to areas suitable to them, many pathogens being unable to survive outside the habitat to which they are adapted. Because of these distribution limitations, plants or products coming from Temperate Zone areas similar in flora, crops and climate to our own involve a much greater risk to us than importations brought from purely tropical regions. This does not mean that importations from tropical lands are always safe; it does mean, however, that the overall danger of introducing pests is considerably less to us from tropical regions than from comparable temperate zone areas.

Regional risk. Regional differences in our own country have also to be considered. Certain disease-carrying products might involve little danger of introduction when imported into Maine, but much when brought into Florida, or *vice versa;* and where domestic movement of such produce is unlikely or can be controlled, quarantine restrictions can be safely modified to allow at least a regionally limited importation.

Seasonal risk. A considerable variation in seasonal risk is also well recognized. Such variation is related either to a dependable freedom of certain products from undesirable parasites during a portion of the year, or to unfavorable climatic conditions for pest establishment during a part of the season throughout which importation may safely be permitted.

Volume of importation. That a rising volume of importation usually implies an increase in pest-introduction risk will be generally admitted. The original quarantine plan for nursery-stock importation restrictions frankly recognized this risk and boldly proposed to lessen the dangers of pest introduction by reducing imports to the minimum which would serve essential national needs (43, 60). Several other aspects of volume relation to risk deserve mention, however. In the first place, it would be incorrect to regard risk as varying directly with volume all through its range. Ordinarily, as importations rise in amount, risk undoubtedly increases, but at some point risk reaches a maximum, so that introduction becomes not a chance but a practical certainty, and further increase in volume of importation has no significance. Naturally the maximum point will vary greatly according to the product and the pathogen concerned. In the second place, no true picture can be obtained of risk relation to volume when it is estimated in reference to a month's or even a year's importations. Risk represents a single disease introduction, equally disastrous to the country whether it occurs this year or two years hence; it has a definitely cumulative aspect and should be computed with due regard to a broad time scale. Finally, risk has to be thought of in some cases in connection with product dilution. When a large shipment is broken up and distributed in small lots, each of these becomes a natural unit for disease establishment purposes. Yet because of spore depletion, mortality, reduction in the chances for finding a host, and, above all, the need for a certain threshold population of the pathogen to assure any reasonable hope of survival (66), the risk in each of the unit lots may drop well toward zero. Product distribution has thus produced a dilution effect where even the combined risks of the numerous small subdivisions rank far below that in the original shipment.

Inspection and treatment. These procedures are by no means free from risk (43, *4–9;* 61). However competently and faithfully carried out, they can not assure complete protection. It is not practically possible to examine large volumes of products such as fruits and vegetables, potatoes and broomcorn, on more than a sample basis, a procedure which allows opportunity for the undetected entry of chance or sporadic pathogens. It is true that repeated examinations of adequate samples may eventually bring these rarely occurring cases to light, but this time lag in detection constitutes a definite risk of disease introduction. Many pathogens can not be recognized with any degree of certainty by routine inspection methods; incidentally carried spores, systemic fungi, bacterial diseases and virus troubles figure prominently in this category. Even when the attempt is made to scrutinize carefully and completely every unit of imported material much is invisible to the inspector's eye. Still further, his work can fall short of perfection because of simple human weaknesses and limitations—work pressure, bad light, cold, fatigue, monotony, to name a few. Treatments could be expected to function with the unvarying fidelity of heat or chemical action, but even here irregularities occur at times and introduce an element of risk. The eternal need for compromise with cost and time and avoidance of injury forces treatment formulas into rigid patterns, usually with small margins of safety and often not flexible enough to cover the wide range of conditions encountered.

Complementary procedures. Some of the risks inherent in importation can be appreciably reduced by the adoption of complementary procedures, a series of two or more safeguard measures each independent of the other and each applying to some specific aspect of the pest problem. The combined value of such a series may be very high, first because of the general overlapping effect, and second because the special efficiencies of the several elements tend to cover the whole field more or less completely. By applying in such series various procedures, no one of which is in itself complete or dependable but each of which makes a definite contribution, it is possible to secure a great over-all reduction of pest risk.

Residual risk. The most drastic exclusion measure that can be imposed is that of embargo. Quarantine effort can go no further than to prohibit entry. Yet even when this ultimate step is taken there still remains a certain chance of disease introduction, a chance beyond our power to prevent, a residual risk. It lies in unforseen contingencies, unexpected happenings, in movements abnormal, wayward, unpredictable. All quarantine effort is subject to this residual risk which must be accepted patiently as something entirely beyond control.

The human element. Finally, a considerable element of risk is introduced into the problem by the unsocial attitudes of a relatively few human beings, who for one reason or another refuse to participate in their country's protection program, largely, we may suppose, because of failure to grasp its true significance.

National cultural development affects disease exclusion effort. Although national or political features do not properly belong in a biological consideration of plant disease introduction, the subject would be incompletely covered if no reference were made to certain relationships existing within the human group which tend to modify introduction possibilities. A nation's plane of culture affects the situation rather definitely. Economic success and a high standard of living create needs and desires for a wide range of imports and provide ample means to pay for these. The more progressive nations are thus likely to import materials in larger volume and in greater variety than less advanced countries, and it is almost axiomatic that both diversity and volume of imported products will entail a greater risk of disease introduction. On the other hand, it may be expected that increased national wealth and cultural progress will be associated with high scientific advancement and effective organization, which should connote a deeper understanding of national interest, an awareness of the danger in pests, and an earnest determination to protect national crop assets against these undesirable plant immigrants.

Conditions in the country of origin likewise affect the situation. A progressive foreign country, by virtue of its greater knowledge, facilities and experience, is in much better position to supply us with reasonably pest-free products, such as we could not hope to obtain from still primitive world sources. A high degree of cultural development in our exporting source also gives promise of an intelligent understanding of our protection problems, a sympathetic attitude of mind toward them, and effective cooperation in their solution.

Disease exclusion and national policy. Within our own country national viewpoints and policy have much to do with our attitude toward disease introduction. National leadership may weigh the value of certain import needs as far exceeding the disadvantages of pests they are likely to bring. There may arise tendencies to modify pest-protection arrangements to accord with international trade programs. And special emphasis on industrial export, which must usually be paid for by imports of foreign agricultural products, could easily result in a vastly increased influx of such products, with pests included.

Effect of disease introduction on export. Another national aspect of plant-disease introduction likely to be overlooked lies in a possible adverse effect on future agricultural exports. The advent here of a new and troublesome disease in a crop normally entering into export may arouse among customer nations a lively fear that continued importation from us would bring the undesirable pathogen to their own agriculture. It is but natural that they should attempt to avoid such misfortune by establishing whatever quarantine barriers are possible, in this way reducing or ending a hitherto profitable export trade. The national loss involved in curtailment of such a trade outlet falls on producers, carriers and business firms directly, but it also affects indirectly an intricate network of dependent individuals and enterprises, and the total may run into huge figures. Economic repercussions from some unfortunate disease introduction can in this way entail serious national losses quite independent of direct damage from the disease itself or the costs of control to growers.

PROGRAM AND PROCEDURES OF DISEASE EXCLUSION

What practical procedures are available to the national authority, acting on behalf of the people as a whole, which would enable the country to exclude dangerous foreign crop parasites? In general, the national protective effort would logically comprise four lines of defense: (*a*) A comprehensive study of the identity and relationships of foreign diseases, as a vital prerequisite to intelligent planning and action; (*b*) the exercise of adequate control over the importation of all pest-carrying materials; (*c*) domestic survey activities designed to detect pathogens which may have unfortunately eluded importation control barriers and which a watchful home organization may discover while they are still in an isolated location or in an incipient stage, possibly amenable to eradication; and, finally, (*d*) a domestic organization capable of prompt and effective action, looking either to eradication or to limiting or delaying the spread of an intruder pending development of control measures.

Knowledge of foreign diseases. The importance of adequate knowledge regarding foreign diseases was stressed long ago by Orton (60), more recently by Hunt (37), and has been brought to attention many times by the American Phytopathological Society (3). Stevenson (74) in 1926 listed the most important plant disease organisms then known to occur abroad on a considerable number of host genera, and annual publications of the Bureau of Entomology and Plant Quarantine (79) record the pathogens currently found on foreign materials examined by its inspection service. Considerable information valuable to the Nation's protection effort has been obtained by various American workers traveling abroad, their contributions representing either the result of specific disease investigations or data acquired incidental to some special research interest. Illustrative of these sources of information are studies of the white pine blister rust in Europe by Spaulding (67); larch canker investigations in England and Scotland by Hahn and Ayers (30); source studies of chestnut blight in China and Japan by Meyer (65); forest pathology notes made by Boyce (10) in Great Britain and Denmark; much information on sugar-cane diseases in Pacific and Asiatic regions collected by Brandes (11); the studies of Weston (88) on downy mildews of corn in the Orient; field notes in Cuba by the author (51) on lima bean scab; studies on scab of sweet orange made by Jenkins (9) in Brazil; data on strain relationships of wheat stem rust built up by Abbott, Stakman and others (1) in Mexico and South America; and reports by Hartley and Rands on plant pathology in Java (32), by Metcalf on chestnut blight in Europe (55), and by Darker (19) on Asiatic diseases.

With this far from complete list of individual contributors must be associated the host of specialists who have assembled regional or monographic compilations in various fields, in number and variety too numerous to be cited here. A vast array of foreign literature is available in our larger libraries, and last but not least the extensive records of the Division of Mycology and Disease Survey of the Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, provide a valuable source of reference to the world's plant diseases.

Finally, it is possible to obtain much accurate first-hand information from competent scientific sources in various foreign countries, and it has been proposed by the American Phytopathological Society (4) that certain State Department or other national representatives abroad could be called upon to supply useful information on pest conditions in the countries where they are stationed.

Information on foreign diseases known to affect specific crops or plant groups abroad and drawn from the above and other sources is usually assembled and presented by the Bureau of Entomology and Plant Quarantine at a public hearing where foreign-plant-quarantine promulgation or revision is concerned with disease problems; the quarantines on rice, sugarcane and coffee (80) provide familiar examples of this practice.

Control over imported materials. The system of quarantine control established over the importation of plants and plant products, although constituting only one element in the suggested fourfold protective scheme, dominates the scene to such an extent that the other elements mentioned may be almost lost sight of in the public mind. In the United States this system provides under law (84) for the power of embargo against specified foreign plant materials, as well as the power to require, for certain items or classes, a regulated type of entry. The embargo action may be partial or complete. It may be effective for the whole world, as in the case of citrus nursery stock (80), or may apply to specified countries and localities only, as exemplified in the Dutch elm disease quarantine and the flag smut quarantine. The extent of the prohibition may vary widely also in regard to the plant materials themselves, in some cases applying to a wide range of plant species or closely related groups, and in others only to a distinct class within a species, such as thin-skinned avocado varieties, or to a specified plant stage, such as mango seeds, or leaves used as packing. Such prohibitions may also be effective only for certain destination areas; commercial importation of unroasted coffee beans into Puerto Rico is not allowed, although this product freely enters the United States mainland; and foreign black currants are admissable only into the 12 States practically free from white pines.

When measures permitting a regulated importation are imposed, restriction on foreign sources, materials and destinations may be set up. In addition, the regulatory procedure may include any or all of the following provisions: Entry is made subject to a permit requirement (84), usually regarded merely as a legal device to insure effective control of the shipments at the time of entry. Certain requirements necessary to simplify and facilitate administration procedures, such as a notice of arrival, notice of shipment, and report on utilization, are often included in regulations. Importation may be restricted to specified ports, in some cases because only these ports have adequate facilities for inspection and treatment, and in others to keep the entry of materials possibly carrying pests away from crop areas where such pests might escape and establish themselves. Regulatory requirements may also apply to the shipment in respect to size, amount, growth stage, packing, presence of debris, soil freedom, containers and marking, or to its routing, storage and utilization. Inspection is an important step in the regulatory process, needed to verify the character of the shipment, to assure that the various requirements are complied with and to determine apparent freedom from pests. Importations are usually made subject to treatment, to be given as a routine feature of entry where plant-disease organisms difficult to detect are likely to be present on shipments, such as of rice straw and bagasse, or to be required only when injurious organisms are found during inspection. Delayed release may be necessary with certain importations, mainly those of propagating type, the plants being held under control for observation during one or more growing seasons to give assurance of their freedom from obscure diseases not detectable at time of entry. Finally, special regulatory features are needed to establish safeguards over the movements of foreign shipments merely in transit to other countries, and to check on the safety of ships' stores and supplies, as well as to adapt the general protective procedure to train and airplane traffic, automobile travel, passengers arriving by ship, train or afoot, and to baggage and parcel post.

The survey function. A service which might be expected to locate promptly incipient outbreaks of diseases of foreign origin does not appear to be specifically provided for in the national protective scheme, although the Division of Mycology and Disease Survey has for many years dealt currently in the Plant Disease Reporter with any new or unusual disease occurrence. In more recent times there have thus been brought to national attention such new domestic disease outbreaks as the bacterial ring-rot of potatoes, magnolia scab, tomato streak, the golden nematode of potato, and azalea blight. In 1943, however, a special survey project (81), undertaken by the Bureau of Entomology and Plant Quarantine in connection with war needs, had as part of its objective a search in and around ports of entry for insects and diseases which might have been introduced under the stress of war conditions, or previously.

Domestic eradication and control of introduced pathogens. Organizations which could assume responsibility for the eradication of isolated disease outbreaks or impose quarantines designed to prevent or delay disease spread within the country would include the Division of Domestic Plant Quarantines of the Bureau of Entomology and Plant Quarantine and various divisions of the Bureau of Plant Industry, Soils, and Agricultural Engineering of the United

States Department of Agriculture, and the regulatory agencies of the various States and territories. The nature of the action to be taken in any given case and also the question as to whether it could be carried out most advantageously under State or Federal responsibility are matters usually decided through conference of representative national leadership. An extremely sensible and helpful step was taken by Congress in 1937 (83) in providing a special fund to be used in suppressing incipient or emergency outbreaks of insects and diseases.

RÔLE OF THE FOREIGN CERTIFICATE

Plant-disease-exclusion programs, both in this country and abroad, have generally included the feature of a sanitary certificate issued by a competent official and based on inspection at the point of origin. The Plant Quarantine Act (84) stipulates that an importation of foreign nursery stock, if originating in countries which maintain an adequate inspection service, must be accompanied by a valid certificate, presumably assuring the freedom of the shipment from injurious insect pests and plant diseases.

The actual value of foreign certification as a means for preventing or lessening the introduction of alien diseases appears to be uncertain, even controversial. Gussow (28) urged, among other suggested reforms, that all nations cooperate by exercising special watchfulness over their own exports so that pathogens or pest-carrying materials will not be sent to other parts of the world, this altruistic purpose to be effected by a control system essentially involving point-of-origin certification. This attitude, which places considerable faith in the efficacy of certification procedure, and which is representative of the views of many others on the subject, is not shared by certain practical-minded quarantine workers. They admit that it seems logical and reasonable to have inspection and certification carried out where both crops and diseases are intimately familiar to the local inspection authorities, and that this type of certification can be successfully utilized where a cleanliness of the tolerance type is satisfactory. But it is emphasized that where permanent and complete disease exclusion is the aim, quarantine procedures must attain a faithfulness and exactitude which can seldom be reached or maintained by such routine certification methods. Furthermore, these doubting Thomases are not convinced of the wisdom of placing a country's vital interests so unreservedly in the hands of a foreign official who, even if motivated by an unquestioned good will, can have no compelling interest in or real responsibility for another nation's welfare. They insist that, in accordance with the old law of "caveat emptor", it is both sounder policy and morally more correct for a nation to retain in its own hands as far as possible the control of its own safety; and they express profound misgivings, based on years of actual experience, concerning the standard of performance to be expected, claiming that few countries could be relied on to provide the accurate and reliable certification which disease-exclusion objectives would undoubtedly demand.

VIEWPOINTS ON DISEASE-EXCLUSION POSSIBILITIES

What are the national possibilities in preventing foreign-disease introduction? There appears to be comparatively little well digested opinion available bearing on the future outlook in this field. One school of thought believes that attainment of any worthwhile national security against the advent of foreign pathogens is a visionary and impractical goal; that the biological difficulties are so great as to constitute an almost insurmountable obstacle to the achievement of adequate protection; that the effort in any case could expect a reasonable degree of effectiveness only by virtue of a complex and costly organization and excessive import sacrifices, quite unendurable by the nation at large; and that the realistic and sensible course is to allow foreign diseases to enter, as they will eventually, it is claimed, and trust to technical ability to control them or adapt agriculture to their presence.

Many other national thinkers, however, do not share this pessimistic attitude. They reason that, naturally protected behind effective continental barriers, any powerful and civilized people ought to be able to control its imports in such a way as to exclude undesirable plant parasites quite as successfully as it strives to do in the case of many dangerous human and animal parasites; that even the far-from-perfect functioning of recently established quarantine systems has apparently been successful in preventing or delaying for many years the introduction of various pernicious pathogens; and that a fuller realization of the cost and consequences of pest introduction will in time inspire a widespread popular demand throughout the world for better protection against introduced pests and create support for more stringent exclusion measures as a course definitely in the national interest. They believe that rapidly increasing knowledge, improved technical methods, and greater experience in administrative procedure are likely to assure for the future a standard of quarantine accomplishment now undreamed of, and that the extraordinary past achievements of human society in its age-old struggle to control and subjugate the the natural world to its own will and purpose distinctly encourages hope for the ultimate success of a serious national effort to exclude injurious foreign plant pathogens.

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THE BOTANICAL REVIEW

Vol. XII March, 1946 No. 3

PHYSIOLOGIC SPECIALIZATION OF THE PARASITIC FUNGI. II[1]

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Investigations of physiologic specialization in plant pathogens, especially those which attack cereals, vegetable crops and other economic plant groups, continue to occupy a foremost place in plant pathology. During the past ten years many workers in various parts of the world have contributed to our knowledge of the extent of host specialization and have emphasized the importance of the recognition of this phenomenon in the practical breeding for disease-resistant varieties of crop plants.

CEREAL RUSTS

Puccinia graminis tritici. In 1934, 127 races of the stem rust of wheat were listed for the entire world. Since then, many additions have been made, a total of 189 being recorded in 1944 (127). New races have been discovered in Poland (34), Germany (50, 53, 74), Italy and Italian East Africa (119, 120), Portugal (21), Australia and New Zealand (166), Argentina, Brazil, Chile and Uruguay (150, 153) and in China (145), as well as additional races recorded by American investigators.

A virulent race has been found on Khapli emmer in Peru (35). It is designated as No. 189 and is very locally distributed, not having been isolated from other parts of South America or elsewhere. Since Khapli emmer has been extremely resistant to other known races of the rust, the new race may be an important one, for, if introduced into the spring-wheat region of the United States, it might be disastrous to the new wheat varieties which depend upon Khapli for resistance.

Interesting changes have taken place in the occurrence of specific races of the stem rust of wheat. Race 56, which was first found in 1928, has rapidly increased, in 1938 about 60% of the stem rust isolates belonging to this race (125). In contrast, race 34 has greatly decreased since 1934. Data from the annual physiologic race surveys made in the United States for more than 20 years have been recorded (129). During 1930–1941, five races—Nos. 17, 36, 38, 49 and 56—ranked first in prevalence. During 1930–1934, races 36, 38 and 49 seemed to be the most important, while in 1935–1939, race 56 ranked first. It is especially emphasized that the increase of race 56 during the period was particularly significant, constituting about 90% of the rust in the Mississippi Basin. The predominance of race 56 in Canada may be one of the factors in the reduction of the cultivation of the Ceres variety of wheat (70). Observations on the prevalent races in southern Mexico have been recorded (130).

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[1] Supplement to article in The Botanical Review 1: 119–137. 1935.

A special study of race 15B, which has been identified in many collections from different parts of the United States, has been made (45). It appears to be a potentially dangerous one, since many of the wheat varieties and hybrids being bred in the Mississippi Valley are susceptible to it, and should the newer hybrids be introduced, it might become prevalent and destructive.

The origin of abnormal rust characteristics through the inbreeding of physiologic races has been investigated (68). Observations have been made on such peculiarities as greyish-brown, orange or white urediospores; decreased vigor of sporulation; decreased virulence; increased sensitivity to high temperatures; inability to produce aecia on barberry; and development of urediospores and teliospores by some strains that have partially lost the ability to produce aecia. The crossing of races and selfing of the hybrid generations may lead to recombinations of pathogenic characters resulting in the formation of new physiologic races (69).

Teliospores obtained on *Agrostis* were used to inoculate barberry, and the aeciospores from barberry infected barley, the culture proving to be race 11 of *Puccinia graminis tritici*. Crosses between this collection and races of *P. g. tritici*, *P. g. secalis* and *P. g. agrostidis* yielded additional races of each of these groups, one of the *tritici* races, No. 160, being different from any previously identified (16).

Two varieties of barley were tested with 19 races of *Puccinia graminis tritici* and one of *P. g. secalis,* both varieties being susceptible to the former and resistant to the latter. Other hybrids and varieties of barley were tested, reacting in the seedling and in the adult stage in the same manner to the races used (63).

The recently described species of wheat, *Triticum timopheevi,* has been reported as very rust-resistant. It has been inoculated with 20 physiologic races, without any infection (94). Its resistance has been transferred to the common winter wheat through hybridization (118). More recently, it is reported that *T. timopheevi* is susceptible to races 15B and 189, the newly discovered virulent race from Peru (44).

Puccinia graminis avenae. A stem rust collected on *Avena fatua* infected 18 species belonging to nine genera, distributed in three tribes of grasses, and the rust culture was identified as a strain or race of *Puccinia graminis avenae.* Rust from *Poa ampla* infected 34 species of grasses in 14 genera and five tribes, this rust also probably belonging to the *avenae* group. However, it did not infect 16 out of 17 varieties of cultivated oats, thus indicating a new physiologic race. A culture from *Agropyron spicatum* infected seven species, representing three genera limited to one tribe of grasses and does not seem to belong to any of the known races, not being related to *Puccinia graminis secalis* or *P. g. tritici* (28).

The reaction of oat varieties in the seedling and maturing stages to five physiologic races—1, 2, 5, 7 and 10—has been studied. Races 2 and 5 had been significant in the stem rust epidemics during 1921–1935 (79). Twelve physiologic races in Canada were recorded during the period 1921–1943, races 1, 2 and 5 being the most prevalent, especially in the three Prairie Provinces where the barberry is practically absent. The effect of environment on the reaction of an oat variety to races 1 and 11 is also described. The type 1 infection in summer is frequently replaced by type X. A temperature of about 80° does not appreciably affect the reaction of White Tartar and Richland to the rust. However, the resistance of other varieties, derived from certain crosses, is broken down (93).

Attention is called to the possible importance of race 8 which, along with race 10, causes heavy infection on Richland, Vicland, Boone, Tama and other varieties of oats which are derived from Victoria x Richland crosses (128). Two races of oat rust are recorded for Argentina, practically all of the local varieties of oats being susceptible. Introduced varieties, as Rainbow, Richland, Iogold, Green Russian and Hawkeye, showed resistance. Various grasses, including species of *Dactylis, Poa, Bromus, Briza* and *Phalaris,* showed some susceptibility to the stem rust (158).

Puccinia triticina. Many investigators have added to our knowledge of specialization in the leaf rust of wheat. In 1933, 53 known races in various parts of the world were recorded (81). By 1942 the number had been increased to 129 (71). Additional races have been found in England, Wales and Portugal (107), Poland (100), various other parts of Europe (163), the Odessa region of Russia (39), the vicinity of Leningrad (97), the Saratoff region (169) and also widely distributed over the U.S.S.R. (101). Specialization has also been recorded in Libya and Abyssinia (121), Brazil and Chile (152, 157), India (87) and Japan (5).

Puccinia glumarum. Additional evidence of specialization of stripe rust of wheat and barley has been supplied. Several races have been reported from various parts of Europe (132, 136), from India (87) and China (23), mostly from the Yunnan Province. Practically all of the native wheat varieties were susceptible to the rust. However, a few foreign introductions were somewhat resistant, and might provide a basis for breeding resistant varieties of wheat and barley. Observations made on the occurrence and distribution of this rust in eastern Harz indicate that *Agropyron caninum* may be an important host for races of the rust which pass over to wheat and barley (6).

Puccinia dispersa. The study of specialization in rusts and other diseases of rye is complicated by the fact that it is difficult to obtain genotypically pure rye varieties for inoculation. Leaf rust causes pustules on some wheat varieties and necrosis or chlorosis on others, these reactions making it possible to differentiate two races among ten monospore lines of the brown rust of rye, using 28 varieties of wheat.

Puccinia coronata. In 1930, 33 specialized races of the crown rust of oats were recorded in Germany (33), and in 1933 the same number of races from the United States and Canada (92). Since then, additional contributions to the specialization of this rust have been published. Seven distinct varieties of *Puccinia coronata* on grass hosts in England, namely, *alopecuri, arrhenatheri, avenae, calamagrostidis, festucae, lolii* and *holci,* have been recorded. Some of these are sharply specialized, while others are not. Four races of the *avenae* group are listed, as well as a new one from Portugal (11). Four races of this rust have been described from Argentina. (151).

OTHER RUSTS

Other species of rust have been investigated from the standpoint of physiologic specialization. Three races of *Uromyces graminis* were obtained from over-wintered teliospores on *Melica ciliata.* A sixth race has been added to the five known races of *U. dactylidis,* also three new physiologic races of *U. festucae* and an eighth race of *U. poae* have been recorded (37, 38).

The flax rust, *Melampsora lini,* has been studied extensively. In 1935, 14 races were differentiated in the United States and Canada (31), and ten additional races in 1940, three of these being obtained from material of South America (32). Four distinct races have been differentiated from collections made in Holland, Sweden and Germany (135), and six races have been listed in Australia (167).

In 1935, two races of the bean rust, *Uromyces phaseoli typica,* were differentiated (47), and in 1939 two collections from Florida and Washington were separated as distinct (22). In 1939, 13 physiologic races of this rust were isolated from the United States and Hawaii, two or more occurring in a given locality, varying in their predominance from year to year (46). Later, an additional strain was described from Hawaii (96). In a more complete investigation, 20 physiologic races have been described, based on the use of seven differential host varieties (48).

Specialized races of other rusts have also been found, *e.g.,* in *Gymnosporangium clavipes* on the apple (91); four specialized races of sunflower rust, *Puccinia helianthi,* on *Helianthus annuus, H. petiolaris, H. tuberosus* and *H. subtuberosus* (10); and two races of *Puccinia iridis* in 1934, one being distinguished by the susceptibility of *Iris fulva* and *I. foliosa,* while the other was unable to attack these species of iris (83). Another race of the iris rust was found on the bearded iris at Berkeley, California (84). Two distinct races of snapdragon rust were found in California, several snapdragons resistant to race 1 proving to be very susceptible to race 2 (171). Breeding experiments in Massachusetts for resistance to snapdragon rust, however, revealed no evidence of physiologic races (168).

CEREAL SMUTS

Oat smuts. Several publications recorded evidence of further specialization in the loose and covered smuts of oats. In 1940, 29 specialized races of the loose smut and 14 of the covered were listed (105). Three additional races of each of the smuts were recorded by another investigator (140). An interesting race of loose smut which was able to infect Black Mesdag oats was described in 1938 (160). More recently, an additional race of loose smut was recorded which was interesting because of its ability to attack the Victoria oat variety which hitherto had been resistant to all known races of both loose and covered smuts (106). It has been suggested that loose smut of oats, *Ustilago avenae,* and the smut on *Arrhenatherum elatius, U. perennans,* were specifically identical. Oat varieties were successfully infected with spores from the grass. Hybrids between the two smuts were made, but tall oatgrass remained resistant to both loose smut from oats and the hybrids between the two smuts (29).

Barley smuts. Two physiologic races of the loose smut of barley, *Ustilago nuda,* one attacking a spring barley and the other a winter variety, have been described (141). Several additional races of *U. nigra* have been listed (72, 137, 139). Further specialization in *U. hordei* has been demonstrated, eight races being found in different parts of the United States (138) and evidence of specialization for races in Alberta, Canada (117). In China (174) five races have been recorded. One of the difficult problems in the covered smut of barley is the development of effective methods for the infection of susceptible varieties.

The occurrence of *Ustilago hordei* and *U. nigra* on forage grasses has been described. The covered smut of barley, *U. hordei,* was collected on *Agropyron cristatum* and *Elymus glaucus jepsoni,* and three collections from these hosts infected Beldi Giant and Trebi barley. Canadian oats, however, was not infected by this smut. It was concluded that the covered smut on the grasses has a close relationship to *U. hordei.* Twenty-five species of *Agropyron, Elymus, Hordeum, Lolium* and *Sitanion* were inoculated, and high infections obtained on many of them. Several grass species were also inoculated with *U. nigra,* and 30% to 50% infection was obtained on *Elymus canadensis, Hordeum nodosum* and *Sitanion jubatum* (24).

Wheat smuts. Additional investigations have been carried out with the two species of *Tilletia* on wheat. In 1936, two races of *Tilletia tritici* were recorded (57), and in 1937, eleven races of *T. tritici* and eight of *T. laevis.* In 1942, five additional races of *T. tritici* and *T. laevis* were noted. Studies of some of these races of the two species were made, recording differences in the size, shape and water-absorption properties of the bunt-ball; the size, echinulation and color of the chlamydospore; the capacity to stunt the host or to stimulate excessive tillering; and the incomplete infection and intensification of the pigmentation of the glumes. One race of *T. tritici* gave rise to a different one, indicating the lack of stability on the part of the races (59).

In Russia, five varieties of *Tilletia tritici* have been distinguished on morphological grounds—dark brown, light brown, typical, whitish-brown and spiculate spores—and also three forms of *T. laevis*—dark brown, light brown and greyish-brown spores, a new variety also being described (122).

In 1935, four races of *T. laevis* and nine of *T. tritici* were recorded in Argentina, three of the latter infecting rye (95). In 1938 several races of *T. tritici* were listed for Australia (15), and five races of *Tilletia tritici* and five of *T. laevis* were recorded in Rumania (114).

A series of resistant varieties of wheat was inoculated with six races of *Tilletia tritici* and three of *T. laevis* (7). No significant increase in the percentage of bunt infection was noted when they were inoculated with their own smut over a three-year period, except in one case which may have been due to a segregation in the smut's capacity for infection. Another observer has recorded a marked decline in the pathogenicity of a physiologic race of *T. laevis* when grown on a susceptible variety over a period of years, results at marked variance from those obtained by others (162). The two species of *Tilletia* on wheat have been hybridized, and distinct races developed among the hybrids (56).

It has also been found that both *Tilletia laevis* and *T. tritici* are able to infect species of grasses in the tribe *Hordeae,* including *Agropyron inerme, A. spicatum, A. trichophorum* and *Sitanion jubatum* (25).

Very little work has been done on the loose smut of wheat. However, four races have been differentiated in Manitoba (40).

Flag smut of wheat. In 1936, five specialized races of flag smut of wheat, *Urocystis tritici,* were recorded in China (176), and in 1945, seven additional ones were listed (177). In the United States two races were differentiated in 1943 (58).

The relations of *Urocystis agropyri, U. occulta* and *U. tritici* have been investigated. Twelve species of grass belonging to the genera *Agropyron, Elymus* and *Hordeum* are more or less susceptible to the flag smut of wheat, and four species of grass are somewhat susceptible to the flag smut of rye—*Agropyron caninum, A. inerme, Elymus canadensis* and *E. triticoides.* Among the collections of grass smut studied, three physiologic races were differentiated. It was further noted that wheat is immune to *U. occulta* and rye to *U. tritici.* One variety of wheat showed slight susceptibility to *U. agropyri,* and the smut obtained was easily propagated on the same wheat variety. These results led to the suggestion that the flag smut of wheat in the United States may have arisen as strains of *U. agropyri,* instead of having been introduced from abroad (30).

OTHER SMUTS

Grass smuts. In 1935, four physiologic races of *Ustilago striaeformis,* each restricted to a single genus of grass, were noted. Under the name of *U. clintoniana,* another closely related smut on *Dactylis glomerata* was recognized as a distinct species (18). A specialized race of *U. striaeformis* on the genera *Agropyron* and *Elymus,* which is apparently seed-borne, has been recorded. High percentages of infection were obtained on several species of *Agropyron, Elymus, Hordeum* and *Sitanion,* some species of which are recorded as new hosts for the smut (26).

Host specialization of the head smuts of grasses has been investigated. These smuts have been described under several specific names, each limited in its host range. It is now recorded that *Ustilago bullata* includes *U. bromivora* and *U. lorentziana.* Collections of the smut from various grasses were used in inoculation experiments, and eight physiologic races were differentiated (27).

Sorghum and millet smuts. Studies made in China in 1937 (173) suggest the existence of two strains or races of the millet smut, *Ustilago crameri,* and in 1944 six distinct races were differentiated (164).

Very few additions have been made to the records of specialization in the loose (*Sphacelotheca cruenta*) and covered (*S. sorghi*) smuts of sorghum. Previously, two races of loose smut and five of the kernel smut had been described (88, 90). Minor morphological differences in a physiologic race of loose smut have been noted (108). A new race of covered smut has been isolated from cultures from sporidia, and is characterized by its ability to infect hybrids from a cross of Hegari-Kafir, highly resistant to the hitherto known races of this smut (149).

POWDERY MILDEWS

In 1937, two additional races of the powdery mildew of barley were described. These races were unable to infect various wild species of the genus *Hordeum* (142). In 1944, three additional races were isolated in Canada, making a total number of seven found in that area (13).

In 1938, additional physiologic races of wheat powdery mildew were identified in Germany (111, 115). Three physiologic races have been described from Argentina, and it was noted that practically all varieties of wheat extensively cultivated in Argentina are very susceptible. However, a few foreign varieties are resistant and may prove useful in breeding work. The various species of wheat—*Triticum durum, T. polonicum, T. turgidum, T. spelta, T. macha* and *T. compactum*—are susceptible. There are both resistant and susceptible varieties in *T. monococcum, T. aestivum* and *T. dicoccum. T. timopheevi* is resistant (159).

A large number of collections of species and varieties of grass have been tested with mildew from eight different grass hosts, and it was found that all of the cultures possessed a wide capacity of infection, attacking species of many genera. The powdery mildew from wheat and barley infected certain grass species, and a culture isolated from *Agropyron repens* infected a barley variety. The point is stressed that there may be a wide range of hosts, as well as sharp specialization within the grass mildew (41).

In 1945, five races were differentiated on species of *Poa,* and evidence of specialization of the oat mildew was obtained (42, 43).

The powdery mildew *Erysiphe cichoracearum,* in 1925 and later, was very destructive on cantaloupes in the Imperial Valley of California. Breeding mildew-resistant melons was undertaken, and a marked degree of success was obtained. In 1938, however, it was found that certain of the new resistant varieties became severely infected, and tests showed that a new physiologic race of the powdery mildew had appeared, severely attacking practically all the commercial varieties. This necessitated an attempt to develop types resistant to the new race (66).

Specialization has been found in other species of mildew. Studies have been made with the powdery mildew of phlox (*Erysiphe cichoracearum*) and some evidence of the occurrence of physiologic races secured (85). The existence of six physiologic races of *Sphaerotheca fuliginea* was demonstrated, these also being separated on the basis of the average length of the conidia (49). Studies in Russia on the same powdery mildew established three distinct races, one limited to *Calendula,* the second to *Bidens* and the third to *Taraxacum* (112).

OTHER PATHOGENS

Many other groups of pathogenic fungi have been investigated, and physiologic specialization has been observed to be of wide occurrence. Physiologic races have been found in fungi belonging to very diverse groups, such as *Bremia lactucae* (64, 116), *Cercospora oryzae* (67, 113), *Cladosporium fulvum* (75), species of *Fusarium* (144), *Ophiobolus miyabeanus* (143), *Phytophthora infestans* (8, 77, 103), *Pseudoperonospora humuli* (55) and *Rhynchosporium secalis* (12).

SUMMARY

Thus the investigations of the past ten years have resulted in the differentiation of many new races among a wide range of plant pathogens. The races may have a very narrow range of host plants, or they may occur on species belonging to different genera.

Some investigations have revealed minor morphological differences between many strains or varieties of pathogens, so that there is no sharp line of distinction between some of these and races, which can be recognized only by experimental tests.

The powdery mildews of peach and rose, *Sphaerotheca pannosa,* seem to differ both morphologically and pathologically, the mildew from one host not infecting the other (172). Morphological varieties have been found in *Tilletia laevis* and *T. tritici* (59, 122).

Further, problems have been raised in connection with the utilization of suitable test varieties. In the crown rust of oats, Straib (134) differentiated many more races by the use of additional host varieties, as compared with the results obtained using the set originally developed by Murphy (92). Kingsolver and Murphy (73) obtained similar results in their experiments. Hassebrauk (54), on the basis of his work with the leaf rust of wheat, suggested that the differential hosts appropriate for one country may not be suitable for another. Rashevskaya and Barmenkoff (101) also found that the so-called standard varieties used for differentiation were inadequate for distinguishing physiologic races of this rust in collections made in Russia. Honecker (60, 61) found the varieties of barley used by Mains and Dietz (86) to be unsatisfactory for differentiation of barley mildew races in Germany.

Based on physiologic tests, many pathogenic fungi usually recognized as distinct species, seem to be distinguished best as physiologic races. There would appear to be no sharp lines of separation between *Urocystis agropyri, U. occulta* and *U. tritici* (30). The head smuts of grasses usually listed under different specific names certainly are closely related (27).

Another problem is raised in some investigations on *Phytophthora infestans.* Black (8) has recorded two strains, one being more virulent on certain hybrids than the other. Lehmann (77, 78) has suggested the existence of eight biotypes or races, recording great differences in their virulence as well as in their distribution. Reddick (102, 103) has discussed the question of the origin of *P. infestans* and some of the problems in breeding for resistance to this organism, having recorded four physiologic races as being known in North America. He also suggested that the virulence of the fungus is enhanced by passage through a resistant host, and thus it might be possible that an almost infinite range of races would develop if suitable intermediate host varieties were produced.

It is evident from many of the investigations reported that there is need for further standardization of methods and the control of environal conditions in relation to both host and pathogen.

Many pathogens may be grown in culture in the laboratory, and isolates may show distinct racial differences. Christensen and Graham (14) distinguished more than 125 cultural races of *Helminthosporium gramineum* Rabh. Utter (148), in studies on cultures of a race of loose smut and another of covered smut of oats, was able to differentiate many distinct types. Further, from hybrids between the two species, he isolated new races combining the morphological characters of the two smuts with new capacities for infecting oat varieties.

In an important and wide-spread crop such as wheat, great changes have occurred in the distribution of specialized races. Race 56 of *Puccinia graminis,* which was first found in 1928, has rapidly increased in the United States and Canada.

The races in one country may differ decidedly from those in another, and varieties of crop plants may escape infection, due to the absence of races which attack them. The importation of new races from one locality to another may result in serious damage to varieties which were considered resistant.

PHYSIOLOGIC RACES IN CEREAL RUSTS

Fungus	Authority		Year	Country	No. of races
Puccinia anomala Rostr.	Straib	(133)	1937	Europe	14
	D'Oliveira	(20)	1939	England, Portugal, Spain	11
	D'Oliveira	(20)	1939	World	30
Puccinia coronata (Pers.) Corda	Frenzel	(33)	1930	Germany	33
	Murphy	(92)	1933	North America (1927–1932)	33
	Brown	(11)	1937	England and Portugal	4
	Straib	(134)	1937	Germany	139
	Waterhouse	(166)	1938	Australia	5
	Vallega	(151)	1940	Argentina	4
Puccinia glumarum (Schm.) E. & H.	Straib	(131)	1937	South America	4
	Straib	(132)	1937	Germany	38
	Straib	(136)	1939	Alps Region, and Bulgaria, Japan	14
	Mehta	(87)	1940	India	8
	Fang	(23)	1944	China	9
Puccinia graminis Pers. *avenae* E. & H.	Verwoerd	(161)	1931	South Africa	2
	Levine and Smith	(79)	1937	U. S.	5
	Waterhouse	(166)	1938	Australia	6
	Hassebrauk	(53)	1939	Germany	7
	Kummer	(74)	1939	Germany	1
	Newton *et al.*	(94)	1940	Canada	11
	Vallega	(158)	1943	Argentina	2
	Newton and Johnson	(93)	1944	Canada	13

Fungus	Authority	Year	Country	No. of races
Puccinia graminis Pers. *secalis* Eriks.	Verwoerd (161)	1931	South Africa	2
	Cotter and Levine (17)	1932	U. S.	14
	Stakman (124)	1935	U. S.	14
	Kummer (74)	1939	Germany	2
Puccinia graminis Pers. *tritici* E. & H.	Verwoerd (161)	1931	South Africa	8
	Dodoff (19)	1934	Bulgària	8
	Stakman *et al.* (126)	1934	World	127
	Tu (145)	1934	China	6
	Sibilia (119)	1936	Italy	2
	Hassebrauk (50)	1937	Germany and Southern Europe	7
	Sibilia (120)	1939	Italian East Africa	17
	Waterhouse (166)	1938	Australia and New Zealand	9
	Garbowski (34)	1939	Poland	7
	Hassebrauk (53)	1939	Germany	11
	Kummer (74)	1939	Germany	5
	D'Oliveira and DeSousa (21)	1940	Portugal	6
	Vallega (150)	1940	South America	7
	Garcia-Rada *et al.* (35)	1942	Peru	1
Puccinia triticina Eriks.	Mains (81)	1933	World	53
	Asuyama (5)	1935	Japan	2
	Goeschele (39)	1936	Odessa	5
	Humphrey *et al.* (62)	1936	World	86
	Rashevskaya and Barmenkoff (101)	1936	U.S.S.R.	13
	Roberts (107)	1936	England and Portugal	10
	Hassebrauk (51)	1937	Europe	11
	Petrusheva (97)	1937	Leningrad	4
	Ralski (100)	1937	Poland	5
	Vohl (163)	1938	Europe	16
	Sibilia (121)	1939	Libya	1
	Sibilia (121)	1939	Abyssinia	3
	Mehta (87)	1940	India	6
	Vallega (152)	1941	Brazil	3
	Yarkina (169)	1941	Saratoff	4
	Johnston *et al.* (71)	1942	World	129
	Hassebrauk (54)	1940	Europe	45
	Vallega (155)	1942	Argentina	9

PHYSIOLOGIC RACES IN OTHER RUSTS

Fungus	Authority		Year	Country	No. of races
Gymnosporangium clavipes C. & P.	Miller	(91)	1939	U. S.	2
Melampsora lini (Pers.) Lév.	Flor	(31)	1935	U. S. & Canada	14
	Straib	(135)	1939	Europe	4
	Flor	(32)	1940	U. S. & So. America	24
	Waterhouse	(167)	1943	Australia	6
	Vallega	(156)	1942	Argentina	5
Puccinia antirrhini D. & H.	Yarwood	(171)	1937	Calif.	2
Puccinia helianthi Schw.	Brown	(10)	1936	Canada	4
Puccinia iridis (DC.) Wallr.	Mains	(83)	1934	U. S.	2
	Mains	(84)	1938	U. S.	3
Puccinia sorghi Schw.	Mains	(82)	1934	U. S.	2
	Vallega	(154)	1942	Argentina	2
Uromyces graminis (Niessl) Diet.	Gäumann	(37)	1940	Germany	3
Uromyces dactylidis Otth.	Gäumann	(38)	1941	Germany	6
Uromyces festucae Syd.	Gäumann	(38)	1941	Germany	3
Uromyces poae Rabh.	Gäumann	(38)	1941	Germany	8
Uromyces phaseoli (Pers.) Wint.	Harter *et al.*	(47)	1935	Calif. & Wash., D. C.	2
	Parris and Matsuura	(96)	1941	Hawaii	2
	Dundas and Scott	(22)	1939	Fla. & Wash.	2
	Harter	(46)	1939	U. S. & Hawaii	13
	Harter and Zaumeyer	(48)	1941	U. S.	20

PHYSIOLOGIC RACES IN CEREAL SMUTS

Fungus	Authority		Year	Country	No. of races
Sphacelotheca cruenta (Kühn) Potter	Melchers	(88)	1933	U. S.	2
	Melchers	(89)	1940	U. S.	2
Sphacelotheca sorghi (Link) Clint.	Melchers *et al.*	(90)	1932	U. S.	5
	Melchers	(89)	1940	U. S.	3

Fungus	Authority		Year	Country	No. of races	
					Laev.	*Trit.*
Tilletia laevis Kühn, and *T. tritici* (Bjerk.) Wint.	Nieves	(95)	1935	Argentina	4	9
	Holton and Heald	(57)	1936	Washington	..	2
	Spangenberg and Gutner	(122)	1936	Russia	3	5
	Rodenhiser and Holton	(109)	1937	U. S.	8	11
	Churchward	(15)	1938	Australia	4	9
	Savulescu and Sandu-Ville	(114)	1938	Rumania	5	5
	Holton and Rodenhiser	(59)	1942	U. S.	10	14
					Ave.	*Lev.*
Ustilago avenae (Pers.) Jens., and *U. levis* (K. & S.) Magn.	Radulescu	(99)	1935	Rumania	4	..
	Vaughan	(160)	1938	U. S.	1	..
	Reed	(105)	1940	World	29	14
Ustilago hordei (Pers.) K. & S.	Tapke	(138)	1937	U. S.	8	
	Yu	(174)	1940	China	5	
	Yu and Fang	(175)	1945	China	9	
Ustilago nigra Tapke	Tapke	(137)	1936	U. S.	2	
	Josephson	(72)	1942	U. S.	8	
	Tapke	(139)	1943	U. S.	7	
Ustilago nuda (Jens.) K. & S.	Thren	(141)	1941	Europe	2	
Ustilago tritici (Pers.) Rostr.	Radulescu	(98)	1935	Rumania and Czechoslovakia	4	
	Hanna	(40)	1937	Manitoba	4	

PHYSIOLOGIC RACES IN POWDERY MILDEWS

Fungus	Authority		Year	Country	No. of races
Erysiphe cichoracearum DC.	Jagger *et al.*	(66)	1938	Calif.	2
Erysiphe graminis (DC.) *avenae*	Hardison	(43)	1945	U. S.	2
Erysiphe graminis (DC.) *hordei*	Mains and Dietz	(86)	1930	U. S.	5
	Tidd	(142)	1937	U. S.	2
	Honecker	(60, 61)	1937, 1938	Germany	9
	Cherewick	(13)	1944	U. S. & Canada	7
Erysiphe graminis (DC.) *poae*	Hardison	(42)	1945	U. S.	5
Erysiphe graminis	Mains	(80)	1933	U. S.	2

Fungus	Authority		Year	Country	No. of races
(DC.) *tritici*	Rosenstiel	(111)	1938	Germany	6
	Schlichtling	(115)	1939	Germany	6
	Vallega and Cenoz	(159)	1941	South America	3
Erysiphe polygoni DC.	Yarwood	(170)	1936	California	2
Sphaerotheca humuli (DC.) Burr. *fuliginea* (Schl.) Salm.	Hashioka	(49)	1938	Japan	6
	Rud	(112)	1938	Russia	3
Sphaerotheca pannosa (Wallr.) Lév.	Yarwood	(172)	1939	Calif.	2

PHYSIOLOGIC RACES IN OTHER PATHOGENS

Fungus	Authority		Year	Country	No. of races
Bremia lactucae Regel	Jagger and Chandler	(64)	1933	U. S.	5
	Schultz and Röder	(116)	1938	Europe	2
Cercospora oryzae Miy.	Ryker	(113)	1943	U. S.	3
	Jodon *et al.*	(67)	1944	U. S.	4
Cladosporium fulvum Cooke	Langford	(75)	1937	Canada	4
Fusarium avenaceum (Fr.) Sacc.	Tu	(144)	1930	U. S.	2
Fusarium culmorum (W. G. S.) Sacc.	Tu	(144)	1930	U. S.	3
Fusarium gramineum Schwabe	Tu	(144)	1930	U. S.	3
Helminthosporium gramineum Rabh.	Arny	(4)	1945	U. S.	2
Helminthosporium maydis Nisik. & Mke.	Ullstrup	(146)	1941	U. S.	2
Ophiobolus miyabeanus Ito and Kuribayashi	Tochinai and Sakamoto	(143)	1937	Japan	10
Phytophthora infestans de Bary	Lehmann	(77)	1937	Germany	8
	Reddick	(103)	1940	North America	4
	Black	(8)	1943	Scotland	2
Pseudoperonospora humuli (Miy. & Tak.) Wils.	Hoerner	(55)	1940	U. S.	4
Rhynchosporium secalis (Ond.) Davis	Caldwell	(12)	1937	U. S.	6
Sclerospora graminicola (Sacc.) Schroet	Uppal and Desai	(147)	1932	India	2

Fungus	Authority		Year	Country	No. of races
Selenophoma bromigena (Sacc.) Sprague & Johnson	Allison	(3)	1945	U. S.	2
Septoria tritici Desm.	Sprague	(123)	1934	U. S.	1
Synchytrium endobioticum (Schilb.) Perc.	Braun	(9)	1942	Europe	2
Thielaviopsis basicola (B. & Br.) Zopf.	Allison	(2)	1938	U. S.	4

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RESPIRATION. II[1]

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INTRODUCTION

When the subject of respiration was reviewed in this journal 11 years ago it was stated that by respiration was understood those processes occurring in every living cell which involve a release of chemical energy utilised partly in building up compounds of higher energy content and in other vital processes. The writer has since pointed out (65) that there are many organs, such as storage tissues and mature fruit, which do not grow but which, nevertheless, respire actively, although no synthesis of compounds of higher energy content nor any other process needing a supply of energy appears to take place in them, and that it would appear that respiration is concerned in some way in the very maintenance of tissues in a living condition. The energy aspect of respiration has been discussed at some length by Wohl and James (76) who divide the energy released in respiration into that required for growth and that required for mere maintenance. As regards the latter they conclude that nearly the whole of the energy released by plants in what they call the mature phase escapes as heat which is useless to the plant. During growth only the energy which is fixed by synthetic reactions is used by the plant, the rest of the energy released in respiration being lost as heat.

In dealing with the energy relations of those reactions which are supposed to occur in respiration they point out that metabolic reactions are of two kinds, those which occur spontaneously and involve a decrease in free energy, and others, which may be called "driven reactions", which are possible only if supplied with energy. This energy supply may come from spontaneous reactions, and Wohl and James discuss a number of metabolic reactions in which linkage of spontaneous and driven reactions may occur.

These writers also discuss the energy relations of anaerobic respiration. Although the decrease of free energy in the reaction $C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2$ is 71.9 cal. per mol., they conclude that all the evidence indicates that in general this energy is useless to the plant.

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[1] Supplement to article in The Botanical Review 1: 249–268. 1935.

In commenting on the discussion by Wohl and James, Boswell (12) points out that of the two kinds of reaction which respiration involves, hydrolytic and oxidative, it is the latter which is the principal source of energy. These reactions are regarded as forming a step-by-step series whereby hydrogen (or electrons) are transferred between the substrate and oxygen. Each reaction in the series has a characteristic free-energy change which determines its position in the sequence of reactions, since it also determines what reaction it may drive or by what reaction it may be driven. There thus results an orderly progress of the respiratory mechanism, the heat liberated being the final manifestation of the free-energy changes of all these successive reactions. (See also 16.)

NOMENCLATURE

The last decade has produced a number of changes in nomenclature. Thus there is a growing tendency to denote the production of carbon dioxide and alcohol by plants in absence of oxygen as fermentation instead of anaerobic respiration, on the assumption that the process is identical with fermentation by yeast. The chief argument in favour of this change is that the process has been shown to occur in presence, as well as in absence, of oxygen, so that the word anaerobic is definitely misleading. On the other hand, there are tissues, such as those of potato tuber, in which, although carbon dioxide is produced in absence of oxygen, very little alcohol, or even none, is recognisable. To the writer it would therefore appear that, apart from its brevity, the term "fermentation" has little advantage over the term "anaerobic respiration" to denote the production of carbon dioxide in absence of oxygen.

It is generally agreed that in the early stages of both aerobic and anaerobic respiration, hexose is broken down to compounds containing three carbon atoms. It has been customary to speak of this breakdown as "glycolysis", but Turner (71) has pointed out that the formation of lactic acid from glycogen, hexoses and methyl glyoxal which occurs in animal tissues and yeast under certain conditions, is widely known as "glycolysis", whereas in plant respiration lactic acid is certainly not formed in any appreciable amount. To avoid confusion in the use of the term "glycolysis" he has therefore suggested a new term, "triosis", to express the production from a hexose, or some phosphorylated product of a hexose, of an unspecified 3-carbon compound other than lactic acid.

Another term which has come much into use during the last ten years is "Pasteur effect" or "Pasteur reaction". This refers to a phenomenon which is frequently, though not always, observed when tissue is transferred from aerobic to anaerobic conditions, or *vice versa*. If in presence of oxygen the products of respiration are carbon dioxide and water and in absence of oxygen they are carbon dioxide and ethyl alcohol, then if the rate of hexose consumption is the same under both sets of conditions, the ratio of carbon dioxide produced under aerobic conditions to that produced under anaerobic conditions will be 3, as an inspection of the equations

$$\begin{aligned} C_6H_{12}O_6 + 6\ O_2 &= 6\ CO_2 + 6\ H_2O \\ C_6H_{12}O_6 &= 2\ CO_2 + 2\ C_2H_5OH \end{aligned}$$

at once makes clear. But in many tissues a ratio of less than 3, and sometimes very much less, has been observed, which means that the presence of oxygen lowers the rate at which sugar breakdown occurs in its absence. This is the Pasteur effect.

One other expression may be noted, namely, "extinction point", a term which appears to be due to F. F. Blackman. In low oxygen concentrations respiratory quotients are observed which are greater than unity and which indicate that both aerobic and anaerobic respiration (fermentation or zymasis) are taking place. As the oxygen concentration is increased the respiratory quotient falls until it reaches a constant value in the neighbourhood of unity, indicating the extinction of the anaerobic component of the respiration. This oxygen concentration is called the "extinction point".

METHODS

Of recent years manometric methods, with which the names of Barcroft, Warburg, Thunberg and Fenn are associated, have become increasingly popular with workers on plant respiration. These methods, with a full account of the precautions necessary in their use, are described in a book (22) which will be found invaluable to botanists using the manometric technique. A paper by Brown (18) may also be consulted.

In the writer's earlier account of respiration in this journal reference was made to the development by Stiles and Leach of the katharometer for the measurement of respiration. In their apparatus the respiration vessel was closed, an arrangement which is often quite satisfactory but which may not be suitable for some researches on respiration. Now a modification of the katharometer for use in an experimental arrangement in which a continuous current of gas passes over the plant material, has been described (6, 7). The actual experimental work carried out was limited to measurements of photosynthesis, but the instrument could be equally well used for work on respiration.

The change in electrical conductivity of a solution of barium hydroxide as a result of absorption of carbon dioxide was used many years ago by Spoehr and McGee as a basis for the measurement of plant respiration. More recently apparatus in which the same principle has been used has been described (57). In it a specially designed conductivity cell is used with an alternating current Wheatstone's bridge in which the alternating current is derived from an oscillating thermionic valve with valve amplification. By means of this apparatus a quantity as small as 0.11 µg. of carbon dioxide could be determined.

A similar apparatus has been described elsewhere (21) in which sodium hydroxide replaces barium hydroxide and in which, instead of measuring the conductivity by balancing the resistances in the arms of the Wheatstone's bridge as the resistance of the sodium hydroxide solution changes with absorption of carbon dioxide, the resistances in the other three arms are kept constant, and the current flowing through the galvanometer is used as a measure of the conductivity. The apparatus is made self-registering by using a recording ammeter for the galvanometer.

The method has been developed more recently by others (49). Their arrangement comprises an oscillator, amplifier and vacuum tube voltmeter working in conjunction with a specially designed absorption tube in which sodium hydroxide is used to absorb the respiratory carbon dioxide. The change in resistance of the solution in the absorption tube is not determined by balancing resistances in a Wheatstone's bridge, but by measuring, by means of a diode vacuum tube voltmeter, the change in the potential across the cell when a constant alternating current of frequency of about 1,000 cycles per second flows through the cell. The circuit is so arranged that the deflection of the associated galvanometer is almost directly proportional to the change in resistance of the solution in the absorption tube. By substituting a strip of bromide paper for the galvanometer scale and introducing into the galvanometer lamp circuit a clockwork time switch that closes the circuit for a few seconds every hour, the apparatus can be made self-recording. With the apparatus used by the designers it was possible to measure quantities of carbon dioxide down to 10 μg., but the sensitivity could be increased by the use of a more sensitive galvanometer.

Lastly, mention should be made of the work of those (58) who have used the principle of the polarograph for the determination of small quantities of dissolved oxygen and shown how it can be used for measuring the respiration of erythrocytes and some other animal tissues. The method has been extended to the measurement of the respiration of plant material by others (23) who, by its use, have measured the respiration rate of a single oat coleoptile.

RESPIRATION DRIFTS

A number of researches have been described in which data on the drift of respiratory activity with time have been obtained, *e.g.,* in Conference pears during storage at 10° C. (40). After gathering, the respiration rate falls until at maturity the rate begins to increase. This is the "climacteric rise" which may reach two or three times the preceding rate. The climacteric rise is followed by a further rise accompanying a breakdown of the flesh of the fruit, and this again is followed by a final fall in respiration rate as the tissue dies. This respiratory behaviour of pears is similar to that previously described by Kidd and West for apples. The respiratory activity of the fruit on the tree is very closely correlated with the protein and acid contents. During storage the climacteric rise appears to be accompanied by rises in the ratio of protein to alcohol-soluble nitrogen and in sucrose content. Determinations of various nitrogen fractions and carbohydrates throughout the ripening and storage period suggest that respiration involves a utilisation of glucose throughout, and of fructose as well after the climacteric phase. They further suggest that glucose units are formed from the alcohol-insoluble residue which includes starch and that fructose units are formed from glucose units during hydrolysis of starch.

The climacteric rise in McIntosh apples has twice been observed (59, 41). In the latter is a detailed study of the respiration of these apples throughout their ontogeny from a fortnight after fruit setting until the death in storage of all apples picked later. For this purpose samples of apples were taken at different dates and their respiration followed until breakdown of the apple was evident. The initial respiration rates of these samples may be regarded as those of apples on the tree and in storage, and indicate a continuous downward drift from the middle of June to just before the usual time of harvesting when the climacteric rise occurs. This is followed by a slow fall in respiratory activity until death occurs. In some of the respiration drifts of the different samples pre-climacteric and post-climacteric rises were also observed.

Krotkov considers the continuous pre-climacteric fall in respiratory activity to be due to a continuous fall in the concentration of the respiratory substrate. When this has reached so low a value that the respiration rate is insufficient to provide sufficient energy for the maintenance of the protoplasmic organisation, either a fresh source of the substrate or an altogether new substrate becomes available, and the climacteric rise results. As the concentration of substrate again falls, so does the respiration rate until death occurs, when the rate becomes too low to provide the energy for protoplasmic maintenance. It is to be observed that the minimum pre-climacteric rate and the final rate just before death are about the same.

The drift of respiration in developing Jonathan apples has been followed (62), using the Barcroft manometric technique, over a period from 23 May to 26 July. Up to 22 June whole fruits were used and after that date a sector of the apple cut out vertically from stem to calyx was used. The effect of cutting was to increase the respiration rate by about 63%. Shaw found the respiratory activity increased rapidly from an average of 28.56 µl. of oxygen per hour per gram of fresh weight on 23 May to a maximum of 71.88 µl. on 30 May, after which the rate fell rapidly to an average of 12.66 on 22 June, and then very slowly. The period of high respiratory activity is that immediately following fruit setting when there is rapid cell division throughout the flesh of the fruit. The respiratory quotient also underwent marked changes, falling from 0.84 to 0.45 during the period 23 May to 22 June and then gradually rising until on 26 July it reached the value of 0.92. The low values may be the result of the utilisation of oxygen in the production of malic acid.

The drift of respiratory activity in a number of vegetables in storage has been followed (60). The material included fruits (peppers, tomatoes, cucumbers), seeds (peas and beans), roots (carrot), tubers (potatoes) and vegetative parts (asparagus, spinach and lettuce). The course of respiration was followed at three temperatures (0.5°, 10°, 24°) for periods up to 60 days. At all the temperatures the respiration rate fell gradually with time, the decline being most rapid during the first few days and more rapid the higher the temperature. This fall in respiratory activity might be due to lowering of the immediate substrate concentration or to other physiological processes associated with ageing of the cells.

Platenius also determined the respiratory quotients for the various vegetables during their storage. For most of them the quotient was in the neighbourhood of unity, although there was a tendency for the quotient to become slightly lower in prolonged storage. Notably lower quotients were, however, observed with asparagus, spinach and potatoes. Determinations of the sugar content of asparagus at harvesting and after three days in storage showed that the total sugar lost could account for only half the carbon lost in respiration. Since asparagus contains no starch and only a minimum quantity of fat, it must be concluded that protein is utilised to a considerable extent in the respiration of this plant. Since protein gives a respiratory quotient of 0.80 to 0.82, the low values of the quotient found for asparagus can be attributed to a utilisation of protein as well as of sugar for respiration. The same explanation can be suggested for the low R.Q. of spinach, but cannot be valid for potatoes where quotients as low as 0.45 to 0.66 were observed with tubers stored at 0.5° C. There is a possibility, described by Platenius as remote, that part of the oxygen absorbed is used in the formation of organic acids.

The respiration drift in a number of whole storage organs, potato and artichoke tubers, and carrot roots, at 25° C. has been followed (20). Typically, the drift exhibits two phases, first a fairly rapid rise to a maximum and then a very slow fall, sometimes to an approximately constant level. The initial rise is attributed to the change from the lower temperature of storage to the higher temperature of experimentation, one of the factors inducing the increase being evolution of carbon dioxide released from solution as a result of the rise in temperature. Some evidence of a seasonal drift was obtained, the respiratory rate of potatoes appearing to fall as the storage season advanced, and rising again later in the season.

This high initial output of carbon dioxide has also been observed (1) in a number of vegetables, including potatoes, on transference from cool storage to a temperature of 22° C. The workers are of the opinion that the high initial carbon dioxide output in these circumstances was not due to any release of carbon dioxide dissolved in the sap, for if this were so the same burst of carbon dioxide should occur in nitrogen, but it does not. They agree, however, that the initial rise is a definite change-of-temperature effect. In general, the respiration drifts of the organs examined by Appleman and Smith were similar to those of the organs examined by Choudhury, the initial high rate being followed by a fall to a lower rate which either remained constant or fell continuously till the end of the experimental period.

A detailed examination of the drift of respiration in barley germinating in the dark has been made (35). With whole grains the workers distinguish five phases in the respiration drift. First there is a phase of rapidly increasing respiration rate corresponding to free embryonic development, lasting at 21° C. for two days. This is followed by a phase in which the acceleration in the rate slows down. By the seventh day the respiration rate reaches its maximum after which the rate falls with increasing rapidity for a further five days (phase 3). The second and third phases correspond, respectively, to the mobilisation of endospermic carbohydrate reserves and to their exhaustion. In the fourth phase the fall in respiration rate ceases to be rapid but proceeds slowly to a minimum; this is held to correspond to the breakdown of other materials, probably protein. On the twenty-second day from the beginning the respiration begins to rise on account of the activity of micro-organisms. As far as the early part of the period is concerned, these results agree with the earlier ones of Barnell (10).

The course of respiration in germinating wheat grains of a number of varieties has been followed and its relationship to water absorption examined (46). Three respiratory stages during germination can be distinguished. The first phase is characterised by a slowly increasing rate which begins soon after the grain is brought into contact with water. This is succeeded by a phase marked by a rapid increase in rate followed by a decrease. Later the rate again increases and follows an approximately uniformly rising course characterising the final stages of germination. The slow initial rise in the respiration rate does not appear to be related to water content and so does not appear to be due to water shortage but must be attributed to some factor other than deficiency of water.

In a second paper Leach (47) describes the effect of age and kernel size on the course of respiration of germinating wheat. No significant effect appeared in the respiratory activity as a consequence of the different storage periods, 6, 18 and 30 months. Kernel size, however, was found to have a very definite effect on the rate of respiration expressed in terms of carbon dioxide output per unit weight of grain, the rate being higher in small kernels than large ones. From this it is to be concluded that the respiratory activity is mainly in the embryo and that the endosperm contributes a relatively small proportion of the total respiratory activity of the grain.

EFFECT OF EXTERNAL CONDITIONS ON RESPIRATION

Temperature. Gane (24) has measured the respiration of unripe bananas at various temperatures between 12.5° C. and 32° C. Over this range he found the value of the respiration was given by the equation $\log R = 0.843 + 0.0348\, t$ where R is the respiration rate and t the temperature. This gives a temperature coefficient (Q_{10}) of 2.23, a value similar to that previously obtained by other workers for a variety of tissues.

Water content. The relationship of respiratory activity to the water content of wheat grain has been examined by Shirk and Appleman (64). Samples of Minhardi wheat grains were soaked in distilled water at 28° C. for different periods of time varying from 0.25 to 5.75 hours, and the total water, freezable water and rate of oxygen absorption determined in each sample of grain. It was found that the respiration rate ran very parallel with the content of freezable water. According to Shirk and Appleman the rate of respiration of grain following imbibition of water decreased rapidly for five to seven hours and then more slowly for almost 20 hours. Throughout this time an equilibrium between bound and free water was being established, the freezable water-content continuously falling. Throughout the period the fall in respiration rate was closely parallel to the fall in the freezable water-content. Later work by Shirk (63) on developing wheat and rye grains confirmed the parallelism between respiration and freezable water.

Oxygen concentration. Results so far obtained on the effect of oxygen concentration on the respiration rate have varied very considerably with different tissues. Choudhury (20) has measured the respiration of whole potato tubers, whole artichoke tubers and whole carrot roots in atmospheres containing quantities of oxygen ranging from 3.5% to 100%. The effect of oxygen concentration was different with each of these organs. With potato variations in oxygen concentration between 6.2% and 100% had no appreciable effect on the carbon dioxide output, but with carrot low concentrations of oxygen (3.5%) in one root caused a lowering in the respiration rate below that occurring in air, in another root it rose above the level in air; in both there was a tendency for the rate characteristic of air to be regained. Concentrations of oxygen above that of this gas in air brought about increases in respiration, the rate being higher the more concentrated the oxygen, so that the highest rate was observed in 100% oxygen. With artichoke the respiration in pure oxygen was the same as in air, but in three concentrations of oxygen below that of this gas in air, 10.7%, 6.7% and 3.5%, the respiration was lowered, the decrease being greater the lower the oxygen concentration.

The difference in the results obtained with these three organs is probably due to two factors, the different rates of respiration in the different tissues and their different capacities for anaerobic respiration. In the potato the observed respiration rates in air were only about a quarter of those found for carrot and a third of those found for artichoke. With potato the lowest concentration of oxygen employed, 6.2%, was sufficient to effect the oxidation of the respiratory substrate at the same rate as that occurring in air; that is, some factor other than oxygen concentration was the limiting one. In artichoke, on the other hand, when the concentration of oxygen fell below that of the gas in air, oxygen concentration did become a factor limiting the rate of oxidation. With carrot it would seem likely that oxygen limited the rate of respiration at still higher tensions, but with this organ the matter is complicated by the incidence of anaerobic respiration (fermentation), which is shown by a rate of carbon dioxide output in nitrogen which may exceed that occurring in air. In artichoke the rate of carbon dioxide output in nitrogen fell decidedly below, and in potato very much below, that taking place in air.

Marsh and Goddard (55) found that the oxygen absorption by thin slices of carrot in concentrations of oxygen from 5% downwards fell progressively with lowering of the oxygen concentration, but that the carbon dioxide output increased so that the respiratory quotient rose from 0.82 in 5% oxygen to 3.5 in 1% oxygen. This is again explained as due to the incidence of fermentation in low oxygen concentrations, and from the data of oxygen absorption and carbon dioxide evolution Marsh and Goddard calculate that in 5% oxygen true aerobic respiration is reduced to 70% to 75% of that occurring in air, and that in 1% oxygen it is reduced to 20% or less.

An observation by duBuy and Olson (23) on the effect of oxygen concentration on the respiration of oat coleoptile submerged in a nutrient solution may be noted here. The fall in oxygen concentration of the solution was determined polarographically, and it was found that with falling oxygen concentration the respiration rate remained constant until a certain low concentration of oxygen was reached, when the respiration rate suddenly fell to half its previous value above this concentration. With young coleoptiles this critical concentration was about 0.45 volume per cent. and for old coleoptiles about 0.25 volume per cent. The significance of this finding is discussed in a later section of this review.

In an investigation which had for its main object the ascertaining of the physiological basis for the different conditions under which wheat and rice flourish, Taylor (66) has examined among other things the effect of different oxygen tensions on the respiration of seedlings of these two species. His results indicate that with both wheat and rice there is a progressive lowering of oxygen absorption as the oxygen concentration is reduced from that of air to zero. As regards carbon dioxide output, on the other hand, whereas in wheat it also falls with lowered oxygen concentration, in rice it appears to increase with decrease in oxygen concentration, and in pure nitrogen the rate of evolution appears to be about 50% higher than in air. These results suggest that the superior capacity of rice over wheat for growth under conditions of poor aeration is related to its ability to respire under anaerobic conditions.

Experiments in which the respiration of rhizomes and leaves of a number of semi-submerged plants were measured in different oxygen concentrations, are referred to later.

Carbon dioxide concentration. A high concentration of carbon dioxide was shown by Kidd many years ago to have a retarding effect on both oxygen uptake and carbon dioxide output by germinating mustard. Livingston and Franck (50), however, in an investigation concerned mainly with photosynthesis, have found this is not always so in leaves of *Hydrangea otaksa.* In leaves taken from the plant in December the respiration rate was about the same in air, in 5% carbon dioxide and in 20% carbon dioxide. Exposure of the leaves in 20% carbon dioxide to light usually depressed the rate of respiration. Sometimes the output of carbon dioxide from leaves respiring in a high concentration of carbon dioxide exceeded the intake of oxygen. This effect is reminiscent of the effect of high concentrations of carbon dioxide on the respiration of apples observed by Thomas to which he gave the name CO_2—zymasis.

Inorganic salts. The relation of inorganic salts to respiration was brought into prominence by Lundegårdh's well known theory of anion respiration based largely on that worker's observation of the parallelism between respiration rate and the amount of anion absorbed by cells from solutions of a number of salts. It is not proposed in this review, which deals primarily with respiration, to enter into the vexed and complex question of the relationships supposed to exist between respiration and accumulation of salts or their anions by various tissues, as the emphasis in the work of most recent investigators of this subject has been rather on the factors controlling salt uptake than on those controlling respiration. It is the intention of the present writer to discuss these problems elsewhere. Some recent observations, however, in which an effect of inorganic salts on respiration has been found, may be mentioned here. In particular, reference may be made to the work of Robertson (61) who examined the intake of various chlorides and the carbon dioxide evolved by thin slices of carrot root, and who concluded that all the chlorides used, namely, those of potassium, sodium, lithium, calcium and magnesium, brought about an initial increase in the rate of respiration above that of tissue maintained in distilled water. With the monovalent chlorides the high respiration rate continued for some 150 to 200 hours, whereas with the divalent chlorides the rate fell off after about a day. Bennet-Clark and Bexon (11) also found that chlorides affected the rate of respiration of thin slices of red beet root. They record a progressive increase in respiration rate of this tissue when it was washed in running tap water. This development of respiratory activity is increased when the disks are kept in 0.05 N potassium chloride or calcium chloride, the effect being greater with potassium chloride than with calcium chloride.

A very marked effect of manganese chloride on the respiration of wheat roots has been observed by Lundegårdh (51). He found that the uptake of oxygen by these organs was increased from 155% to 470% by addition to the medium of 0.00005 M manganese chloride. In contrast to this, ferric chloride or ferric citrate brought about a small decline in respiration rate, the mean reduction produced by 0.00005 M ferric citrate being about 21%. Lundegårdh concluded that manganese, but not iron, was a catalyst in aerobic respiration.

An examination of the effect of ammonium chloride and a few other salts on the respiration of shoots of *Elodea canadensis* kept in the dark has been made by Jones (38). He concludes that these generally bring about an increase in the rate of respiration, but that the effect is transitory. Both in water and in salt solutions there is a fairly rapid fall in respiration to a low rate, which is related to a loss in the healthy appearance of the shoots. This behaviour is not related to limited carbohydrate supply, since the course of events is essentially the same when the shoots are pretreated with glucose or sucrose. When, however, the shoots are put in a solution of 0.05% asparagine the onset of the low respiration rate is delayed and the shoots retain their healthy appearance, while the same is the case with a solution of 0.01 M ammonium chloride + 0.05% asparagine. In the latter solution the respiration of *Elodea* also exhibits the transitory rise due to the presence of the salt. These results are interpreted by Jones as indicating that the fall in respiration rate and loss of health is related to a disturbance in the nitrogen relations of the tissue, and it is suggested that the effect of salts or prolonged darkness is to bring about an excess of protein degradation over synthesis with consequent loss of vitality.

Ethylene. Gane (24) treated bananas with ethylene in the preclimacteric phase of low respiratory activity. This treatment always brings about immediately the rise in respiration rate associated with ripening of the fruit. Gane finds that ripe bananas themselves produce ethylene and that the acceleration in ripening of the fruit produced by ethylene in a concentration of one part per million is similar to that produced by the metabolism of the ripe fruit. The conclusion is drawn that ethylene, as a normal product of metabolism during the climacteric, acts as an autocatalyst.

RESPIRATORY ACTIVITY OF DIFFERENT TISSUES

Some recent observations have been made on the different respiratory activities of different parts of the same plant or organ. Goodwin and Goddard (27) have made such observations on tissues isolated from the wood of ash and maple in early spring before opening of the buds. They find that respiration is then most active in the cambium and somewhat less active in the adjoining secondary phloem and xylem. Oxygen uptake becomes progressively less in passing from the cambium to the middle of the tree. In the heart-wood there is a low rate of oxygen absorption which is not destroyed by boiling and which is probably to be attributed to the slow oxidation of organic substances in the dead cells. This also occurs in phloem and sapwood where it contributes a little to the total oxygen consumption. After opening of the buds the respiration of cambium, phloem and sapwood of the ash is about the same as before, but the respiratory activity of the newly formed and differentiating xylem considerably exceeds that of the cambium.

Machlis (53) has measured the respiration rate of portions of barley roots cut at different distances from the root apex, and, as might be expected, found that the respiratory activity decreased with increasing distance from the apex.

RESPIRATION OF PLANTS OF VARIOUS TYPES

Semi-submerged plants. The respiration of plants possessing rhizomes which live in submerged mud presents a special problem in regard to their respiration, for their conditions of life are almost anaerobic. A special study of this problem has been made by Laing (42, 43, 44).

It may be noted at the outset that the oxygen content of the internal atmosphere in the rhizomes of these plants may be very low. Laing analysed the internal atmosphere of petioles, culms and rhizomes of a number of semi-submerged plants belonging to the following species: *Nuphar advenum, Peltandra virginica, Pontederia cordata, Typha latifolia, Sparganium eurycarpum* and *Scirpus validus.* In *Nuphar advenum,* for example, the oxygen concentration of remote parts of the rhizome can fall as low as 0.6 volume per cent., although during the afternoon of a sunny day in summer the oxygen content can rise as high as 7%. Rather similar low values were also observed in *Peltandra virginica, Sparganium eurycarpum* and *Scirpus validus.* The concentration of carbon dioxide, on the other hand, can rise to remarkably high levels, values between 10% and 20% being common, while in submerged young rhizomes of *Typha latifolia* values of 27.5% and 38% were recorded. Measurements of the respiration by a Pettenkofer method of the rhizomes and leaves of the plants mentioned above and of others growing in a similar habitat were made in which the organs were exposed to air, nitrogen and various gas mixtures containing oxygen in concentrations ranging from 0.1% to 10%. It was found that the rhizomes of all the plants examined were able to respire anaerobically in nitrogen for several days without appreciable injury. The rhizomes of *Nuphar advenum* appear to be particularly resistant to injury from anaerobic conditions. The rate of evolution of carbon dioxide was, however, somewhat lower in nitrogen than in air. On the whole, the respiration rate in the gas mixtures decreased with decrease in the oxygen concentration, although numerous exceptions to this occurred; it is not clear to what extent these exceptions are due to variations in the material used. With leaves the results were different. It was found that young leaves of *Nuphar advenum* and the leaves of *Typha latifolia* could respire anaerobically for some days, although the initial rate was not maintained over this length of time, the rate falling to about half in four days. With older leaves of *Nuphar advenum,* however, the capacity to respire anaerobically was very much less, the rate of carbon dioxide output at the end of four days being only about 6% of the original.

✓ The general conclusion may be drawn from this work that the rhizomes of semi-submerged plants respire both aerobically and anaerobically, the extent to which one or other of these processes predominates varying with conditions, particularly with those which induce photosynthesis in the leaves and the consequent setting up of oxygen and carbon dioxide diffusion gradients in the internal aerating system throughout the plants.

Arctic plants. Wager (75) has measured the respiration rates of a number of arctic plants at temperatures from 0° to 40° C., and has calculated the temperature coefficients (Q_{10}) for the four temperature intervals 0°–10°, 10°–20°, 20°–30° and 30°–40°. In general, the coefficient falls with rising temperature. Thus for shoots of *Cassiope tetragona* the coefficients were 3.3, 2.9, 2.2 and 1.7, over the intervals 0°–10°, 10°–20°, 20°–30° and 30°–40°, respectively. For leaves of *Antennaria alpina* the corresponding values of the temperature coefficients were found to be 4.2, 3.3, 2.0 and 1.6, and for shoots of *Saxifraga oppositifolia* 3.0, 2.7, 2.2 and 1.9. Wager made determinations on both winter and summer plants, the species used in the two sets of experiments being different. He found the average Q_{10} of the respiration rate of the summer plants was less than that of the winter plants (2.3 as against 2.7 for the 10°–20° interval). This is attributed to a higher substrate concentration in the plant cells in winter, support for which idea is found in the higher osmotic pressure of the plants in winter, presumably due to a greater concentration of soluble carbohydrates. Wager also concludes that arctic plants respire at a higher rate than those of temperate regions.

Succulent plants. Two contributions dealing with acid metabolism and respiration of succulent plants have been published by Thoday in collaboration with Jones (Mrs. K. M. Richards) (67, 68). The species examined were two succulent species of the Compositae, *Kleinia articulata* and *K. radicans.* As usual with succulents, the plants contain much malic acid. In both species there was observed the familiar diurnal fluctuation in malic acid content, this falling during the day and rising during the hours of darkness. During prolonged starvation of the stem of *K. articulata* in continuous darkness there is loss of both malate and soluble calcium, suggesting that the malic acid associated with calcium is removed and that the calcium is deposited in soluble form. The respiration rate of the stem falls rapidly during the first few days of starvation and is then maintained at a fairly constant level for a variable number of days until a rapid rise occurs corresponding to injection of the intercellular spaces with cell sap. Fluctuations in respiration rate during the first few days of starvation, such as were observed by Bennet-Clark in the Crassulaceae, were not recognisable with *K. articulata,* but this might have been due to lack of uniformity in the material, since with the relatively more uniform material of *K. radicans* the starved leaves in summer showed diurnal fluctuations of carbon dioxide output very similar to those observed by Bennet-Clark in the leaves of *Crassula lactea.* This first phase of starvation was followed by a second one lasting about four days in which respiration rose and then fell. This again was followed by a third phase characterised by a very rapid rise in respiration to a maximum higher than that reached in the earlier phases, and then a less rapid fall. The last phase coincided with that injection of the intercellular spaces with sap which had previously been observed also in *K. articulata.* The three phases are not always distinct, for they may exhibit some overlapping. The third phase is reminiscent of the observation of Bennet-Clark and Bexon, mentioned later, of the increased rate of respiration which occurs when thin slices of beet root are transferred from water to juice expressed from the beet root, and Thoday and Richards regard it as highly probable that the last phase in the respiration of starved *Kleinia,* corresponding with injection of the intercellular spaces, is an example of the same phenomenon.

EFFECT OF MECHANICAL STIMULATION ON RESPIRATION

A previously unsuspected factor affecting the respiration rate of leaves has been described and investigated by Audus. This factor is mechanical stimulation. While examining the course of respiration of leaves of cherry laurel (*Prunus laurocerasus*) over a prolonged period, Audus (2) found that if leaves were removed from the experimental chamber for a few minutes during the course of an experiment, their respiration on replacement in the chamber was considerably greater than immediately before their removal. Further experiments showed that this rise was unrelated to any change in temperature or humidity to which the leaves were exposed, nor was it due to any short exposure to light. The rise in respiration rate must be attributed to the handling the leaf had suffered. The same effect can be produced by stroking or bending the leaf by a mechanical arrangement in the plant chamber, so that the leaves are not subjected to any change of conditions other than the bending. Audus later (3) extended his observations to leaves of 15 other species, including *Laurus nobilis, Rhododendron Fargesii, Yucca gloriosa, Hedera helix, Sparganium ramosum, Typha latifolia, Polygonum cuspidatum* and *Pelargonium zonale.* The effect was noted with all of them, the observed percentage increase in the respiration rate varying from about 20% to 183%. It is important to note that Audus found (4) that in an atmosphere of nitrogen mechanical stimulation has no effect on the rate of carbon dioxide output. He concludes from this that the stimulation does not bring about an increase in the concentration of respiratory substrate, but must affect the oxidation process itself.

Later Audus (5) examined the effect on respiration of two successive stimulations and of a series of stimulations at short intervals of time. He found that the second stimulation of a pair always produced a very definite response, but although the respiration rate reached as a result of the second stimulation may be actually higher than the peak reached after the first stimulation, the net increase in the respiration rate after the second stimulation is always less than that resulting from the first stimulation if the time between the stimulations is less than 90 hours.

Audus could explain his results by supposing that the sensitivity of the leaf to stimulation is lowered by a previous stimulation. Normal sensitivity is subsequently recovered, but much more slowly than the normal respiration rate. A series of stimulations at short time intervals does not appear to reduce the sensitivity of the leaf below that resulting from the first stimulation.

Increase in the respiration rate as a result of handling has also been observed in cherry laurel leaves (26) and in potato tubers (9).

MECHANISM OF RESPIRATION

For many years the Pfeffer-Kostytchev view of the course of hexose breakdown in respiration has been accepted by most plant physiologists. As is well known, according to this view there is an intimate connexion between aerobic and anaerobic respiration or fermentation, the first stages in the two processes being the same and consisting in the breaking down of the hexoses to intermediate products which in presence of oxygen are oxidised to carbon dioxide and water, and in absence of oxygen are transformed to other products, usually carbon dioxide and ethyl alcohol. The first stages common to both processes are now known as glycolysis, or triosis as Turner would prefer this degradation called.

In 1930 Lundsgaard proposed abandonment of the Pfeffer-Kostytchev theory of the intimate connexion between aerobic and anaerobic respiration, as a result of his experiments on the different effects of monoiodoacetate on these two processes. Turner (73) has since made an investigation on the effect of sodium monoiodoacetate on aerobic and anaerobic respiration of carrot root tissue and finds that the iodoacetate inhibits both processes in the same way, although the rate of inhibition of aerobic respiration is less than that of anaerobic respiration. This Turner ascribes to the influence of oxygen in reducing the effectiveness of iodoacetate as an inhibitor of glycolysis in living tissue. It may therefore be concluded that the different effects of monoiodoacetate on aerobic and anaerobic respiration are not discordant with the generally accepted theory of the connexion between aerobic and anaerobic respiration.

The mechanism of glycolysis has been made a subject of special study by James and a number of collaborators. With Norval (37) he had shown that pyruvic acid might be a normal intermediate, that is, a product of glycolysis, in barley, by adding killed young barley tissue to pyruvic acid (M/10 or M/15), with the result that the latter was broken down with formation of carbon dioxide and acetaldehyde on account of the action of carboxylase. Further evidence of the formation of pyruvic acid in barley respiration was later obtained (35) by poisoning roots of barley plants with 0.01% to 0.1% solutions of acetaldehyde or 0.3% solutions of certain aromatic sulphonic acids (35). Unlike untreated roots those so treated gave an ammonia-nitroprusside reaction for pyruvic acid. Moreover, from cut leaves in the dark treated with 0.2% 1-naphthol-2-sulphuric acid, pure pyruvic acid was isolated as the 2,4-dinitrophenylhydrazone. The poisons used inactivate carboxylase and so would induce the accumulation of pyruvic acid if this were formed as an intermediate product in respiration. Further evidence for the presence of carboxylase in barley was obtained by showing that it could be separated into two fractions, an insoluble apoenzyme (protein) and a soluble co-enzyme (19).

James and Arney (29) have examined the relation of the various phosphorus compounds in barley seedlings to their respiration. The phosphorus compounds were classed as inorganic, esterified and residual. The latter, consisting mainly of phospholipoids and phosphoproteins, were determined from the difference between total phosphate and inorganic + esterified phosphate. Barley grains were germinated in respiration vessels supplied with a culture solution the phosphate content of which varied in the different vessels. Respiration was determined by the Pettenkofer method. Over the range of phosphate concentrations (0 to 0.5 g. $CaH_4(PO_4)_2H_2O$ per litre) used, no effect of this concentration on respiration was observed with whole barley grains, but sometimes the rate of respiration of embryos dissected out from endosperm was increased with increase in the concentration of phosphate. This behaviour is attributed to the presence of much phosphate in the endosperm. With addition of 4% sucrose to the culture medium, however, the respiration rate increased progressively with increase in the phosphate concentration, thus indicating that with adequate carbohydrate supply the phosphate supply can limit the rate of respiration.

In these experiments of James and Arney carbon dioxide output reached its maximum at about the fifth day from germination. At this stage various enzyme systems (amylases, invertases and carboxylase) are in excess, and with abundant sugar it is not surprising that phosphate may then limit the respiration rate. A strong correlation was found at this stage between the phosphoric ester content and respiration. With embryos dissected from the grains the phosphate ester content was found to diminish between the second and fourth days after germination, and the respiration rate diminished along with the fall in phosphate ester content. The work of James and Arney thus indicates a relationship between esterified phosphorus and respiration.

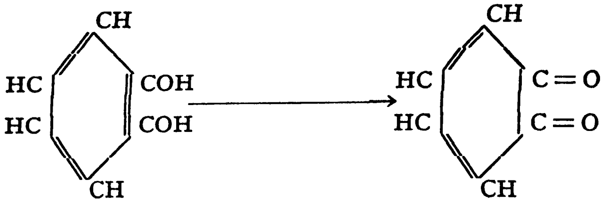
The nature of this relationship was made clearer by other work (36). Cell-free barley sap, which would contain hexose, when incubated at 30° for 48 hours in presence of thymol and 1-naphthol-2-sulphonic acid produced little or no pyruvic acid, even with added sucrose. Addition of adenylic acid to sap low in sugar content brought about the production of what appeared to be traces of pyruvic acid, but when both sucrose and adenylic acid, or both glucose and adenylic acid, were added to the sap pyruvic acid was formed in readily identifiable amount, while addition of hexosediphosphate or phosphoglycerate also brought about formation of pyruvic acid. James and Bunting (30) further found that addition of adenylic acid to barley sap containing sugar and free phosphate induced a considerable increase in the production of carbon dioxide; they also found that hexosediphosphate and phosphoglycerate as well brought about increase in carbon dioxide production. From all this work the scheme shown below is suggested as representing the mechanism of glycolysis:

$$\begin{tikzcd}
H_3PO_4 + \text{adenylic acid} \arrow[d] & & \text{sucrose} \arrow[d] \\
\text{adenyl phosphate} + \text{pyranose} \arrow[d] & & \text{fructofuranose} \arrow[ll] \\
\text{adenylic acid} + \text{hexosediphosphate} \arrow[d] & & \\
\text{phosphoglycerate} \arrow[d] & & \\
H_3PO_4 + \text{pyruvic acid} \arrow[d] & & \\
\text{acetaldehyde} + CO_2 & &
\end{tikzcd}$$

Thus James's view of glycolysis is that phosphorylation of hexose takes place by means of a phosphate carrier which he describes as a labile ester (adenyl phosphate in the above scheme), from which the phosphate is transferred to the hexose to form resistant esters (hexosediphosphate and phosphoglycerate) which are then degraded to pyruvic acid with reformation of phosphate and then, by the action of carboxylase, to acetaldehyde and carbon dioxide.

Although normal respiration is an oxidation process and although a number of oxidising enzymes have been recognised in plants, little was known until recently of the connexion between such enzymes and respiration. From a study of the action of one of these enzymes, catechol (or polyphenol) oxidase, in potato tubers, Boswell and Whiting (14) have come to the conclusion that this enzyme is concerned in respiration of the potatoes.

As is well known, catechol oxidase brings about oxidation of catechol and other substances with the catechol grouping, probably with the formation of *o*-diketoquinone:



Boswell and Whiting found that addition of a small quantity of 0.04 M catechol to slices of potato tuber brought about a considerable increase in their respiration, in both oxygen absorbed and carbon dioxide evolved, an increase which was followed by a fall to a level considerably below the initial rate. This fall is attributed to the oxidation product of catechol inhibiting the action of the oxidase, a view put forward in 1934 by Richter from experiments *in vitro,* and confirmed by the finding of Boswell and Whiting that addition of a further quantity of catechol has no further effect on the respiration rate. It is to be noted that the residual respiration rate depends on the thickness of the tissue slices, being lower the thinner the slice, a result which can be explained on the ground that the thicker the slice the smaller the proportion of cells in the slice which are reached by the catechol. In slices only two cells thick it may be assumed that catechol will diffuse into every cell. With such slices the residual respiration rate after inhibition of the catechol oxidase is 33% of the original rate. From these results it is concluded that two-thirds of the normal respiratory activity of the potato tuber is dependent on the catechol oxidase, while the remaining one-third is dependent on a system not involving catechol oxidase. It may be noted that potato tubers do not contain catechol, but Boswell and Whiting extracted from potato tubers a gummy material containing a phenolic *o*-dihydroxy compound which when added to potato slices brought about a rise in respiration rate. This, after a short time, remained constant at a value about 20% above normal. It would thus appear that potatoes contain an enzyme capable of oxidising phenolic *o*-dihydroxy compounds in the tissue. That this is the same enzyme as that which oxidises catechol is shown by adding the *o*-dihydroxy compound to tissue slices after the catechol oxidase has been inhibited by catechol, when no increase in respiration occurs. It is to be observed that the absorption of oxygen which takes place on the oxidation of catechol and of the *o*-dihydroxy compound in the tissue is accompanied by a production of carbon dioxide. It may therefore be concluded that the oxidation products of these compounds take part in some system which results in carbon dioxide production. This system, according to Boswell and Whiting, involves a dehydrase which catalyses a transference of hydrogen from a hydrogen donator to the oxidised phenolic compound which is thereby reduced to the original *o*-dihydroxy compound. It is supposed that in this action an intermediate compound is formed which splits off carbon dioxide with great ease. Thus on the view of Boswell and Whiting about two-thirds of the respiratory activity of potato tubers is brought about through the action of a system involving catechol oxidase, a phenolic compound with the *o*-dihydroxy grouping, and a dehydrase. The oxygen absorption is involved in the oxidase action, the carbon dioxide evolution in the dehydrase action.

The remaining third of the respiration is related to some system not involving catechol oxidase. Since the respiratory quotient of the whole respiration is unity, and that of the respiration connected with catechol oxidase is also unity, it follows that the respiratory quotient of the other components of respiration is also unity.

The experiments of Boswell and Whiting have been repeated by Baker and Nelson (8) who confirm the results obtained by the addition of catechol to potato slices but deny that the fall in oxygen uptake to a value below that characteristic of the tissue alone is due to the inactivation of the oxidase by catechol, since an even more pronounced fall in oxygen uptake occurs with 4-tertiary butyl alcohol which they say hardly inactivates the enzyme at all. It should be noted, however, that this last finding refers to the polyphenol oxidase from the mushroom. They therefore consider that the claim of Boswell and Whiting that two-thirds of the respiration of potato tuber depends on the oxidase loses its main support. Baker and Nelson describe experiments in which they used protocatechuic acid (4-carboxycatechol) in place of catechol. With this there results a rise in both oxygen uptake and carbon dioxide evolution so that the respiratory quotient remains close to unity, indicating a genuine rise in respiration rate, while very little quinone is produced. These facts suggest that the protocatechuic acid acted as a hydrogen carrier, being oxidised to a quinone by catechol oxidase, the quinone being then reduced by a hydrogen donator. Inhibitors of catechol oxidase, such as potassium cyanide and 4-nitrocatechol, reduced the rate of oxygen uptake of potato slices by about 85%. That the reduction is so great is considered by Baker and Nelson to suggest that probably all the respiration of potato tissue is dependent on catechol oxidase. Boswell (13), however, in a later paper states that the proportion of the respiration due to catechol may vary according to the condition of the tissue, and would explain the difference in the value of two-thirds found by Boswell and Whiting and of 85% found by Baker and Nelson, as due to differences in the tissue used by the two pairs of investigators.

In his later work Boswell examined the oxidation of several other substances in presence of potato tuber tissue; these included a number of phenolic compounds, dihydroxymaleic acid, ascorbic acid and other organic acids, including amino-acids. Among the phenolic compounds he found that homocatechol and *p*-cresol, like catechol, inactivate polyphenol oxidase, while caffeic acid is similar in its behaviour to the polyphenol occurring in, and extracted by Boswell and Whiting from, potato tubers, there occurring a permanent increase in the rates of both oxygen intake and carbon dioxide output when it is added to the tissue. The permanence of the increases, according to Boswell, suggests that the caffeic acid takes part in a cyclic system, that is, it is alternately oxidised (when oxygen is absorbed) and reduced (when carbon dioxide is evolved). If, however, the respiratory activity of the tissue has been first partially inhibited by addition of malachite green, addition of caffeic acid brings about complete inhibition of excess carbon dioxide. This would suggest that the carbon dioxide output results from an action following an oxidation involving a dehydrase, since malachite green is an inhibitor of dehydrases. It may thus be supposed that the oxygen is absorbed in the oxidation of caffeic acid to its quinone by polyphenol oxidase and that carbon dioxide is evolved in the reduction of the quinone to caffeic acid by a hydrogen donator in a reaction catalysed by a dehydrase. Dihydroxyphenylalanine and gallic acid would also appear to form similar redox systems with polyphenol oxidase.

It will be recalled that Boswell and Whiting concluded that polyphenol oxidase controls the major part of the respiration of potato tissue, a minor part of it being controlled by some other system. In regard to this latter, Boswell could find no evidence of the existence of a dihydroxymaleic acid oxidase forming a cyclic redox system, comparable with the polyphenol oxidase system, such as has been suggested by Szent-Györgyi, while as regards ascorbic acid, the conclusion drawn from its effects on oxygen uptake and carbon dioxide evolution was that it might be the co-enzyme of a redox system not directly involving oxygen. It was indeed found that addition of ascorbic acid to potato tuber tissue brought about a temporary rise in oxygen intake and a rise in carbon dioxide output followed by a depression, but this effect is insensitive to both sodium azide and malachite green, from which it is concluded that the oxidation is non-enzymic and metal-catalysed, probable at the surface of the tissue. More interesting results were obtained when various organic acids were added to potato tuber tissue. While the amino-acids glycine, glutamic acid and aspartic acid were without effect on either oxygen intake or carbon dioxide output, both these were increased by addition of succinic, fumaric, *l*-malic, oxalacetic, pyruvic, citric and lactic acids, and Boswell suggests that there may be a progressive oxidation following the course succinic → fumaric → malic → oxalacetic by successive action of dehydrases, for when the first three of these acids are added to tissues in which dehydrase action has been inhibited by malachite green no excess oxygen intake or carbon dioxide evolution occurs.

The decomposition of oxalacetic acid, presumably to pyruvic acid and carbon dioxide, may not be enzymic, since it is unstable and readily decomposed in the presence of proteins. As a result of work on somewhat different lines which will be discussed later, Bennet-Clark and Bexon conclude that in beet root also malic acid is broken down through oxalic acid to pyruvic acid and carbon dioxide by dehydrase action on the malic acid and subsequent decarboxylation of the pyruvic acid produced.

Boswell also examined the effect on the intake of oxygen and output of carbon dioxide by potato tissue first treated with one of the organic acids listed above and then with caffeic acid. He found that pre-treatment with an amino-acid brings about an increase in both oxygen absorbed and carbon dioxide evolved as a result of adding caffeic acid, whereas pre-treatment with a non-nitrogen-containing organic acid brings about a reduction in the excess oxygen absorption and carbon dioxide evolution resulting from addition of caffeic acid. It has been shown that polyphenol oxidase in presence of a suitable phenol can oxidise certain amino-acids, and Boswell concludes that in respiration amino-acids are related in this way to polyphenol oxidase. Boswell's conclusions may be summed up as follows. The respiratory activity of potato tuber tissue depends on more than one oxidase system. The greater part depends on polyphenol oxidase, and this is limited by the amount of polyphenol present in the cells. Hence addition of a polyphenol such as caffeic acid increases the rate of respiration, and when polyphenol is present in excess the supply of the hydrogen donator which reduces the oxidised polyphenol (quinone) back to polyphenol becomes the limiting factor. The amino-acids act as such hydrogen donators, being themselves thereby oxidised, and so bring about an increase in the respiratory activity when caffeic acid is added in excess. Part, at least, of the respiration of potato tuber not controlled by polyphenol oxidase is associated with dehydrase actions in which non-nitrogenous organic acids such as succinic, fumaric and malic may be concerned.

Marsh and Goddard (54) also conclude that two oxidising enzymes are concerned in the respiration of root and leaves of carrot; cytochrome oxidase or a similar enzyme is responsible for most of the respiration in root and young leaves, while one insensitive to poisoning by cyanide, azide and carbon monoxide is responsible for a small part of the respiration in root and young leaves and all the respiration of mature leaves.

Respiration of the cortex of carrot root in the form of slices 0.5 mm. thick was reduced by potassium cyanide, the rate remaining practically constant after a preliminary fall, the reduction increasing from 8.7% with 1×10^{-5} M cyanide to about 80% with 3×10^{-4} M cyanide. No further significant lowering in the amount of reduction was observed with concentrations up to 1×10^{-2} M cyanide, thus suggesting that 80% of the respiration is connected with an enzyme system poisoned by cyanide. A similar inhibition of respiration of carrot root is produced by carbon monoxide, this inhibition being reversed by light. The ratio of residual respiration to respiration inhibited is proportional to the ratio of the partial pressures of oxygen and carbon monoxide; the constant in the relation residual respiration/respiration inhibited = $K \cdot P_{O_2}/P_{CO}$

is found to be of the same order as that found by Warburg for yeast. Reversible inhibition was also obtained with 10^{-3} M sodium azide.

These reactions to cyanide, carbon monoxide and azide all suggest that 80% of the respiration of carrot root is connected with cytochrome or indophenol oxidase or a similar enzyme, and not to catechol oxidase, since the inhibition of this enzyme by carbon monoxide is not reversible by light. Young carrot leaves show a similar behaviour towards cyanide, azide and carbon monoxide, but in mature leaves no inhibition of respiration was effected by these substances, from which it is concluded that some enzyme system other than cytochrome oxidase or catechol oxidase must be concerned in the respiration of mature leaves.

Henderson and Stauffer (28) examined the effect on oxygen uptake by excised tomato roots of a number of respiratory inhibitors, namely, malonate, iodoacetate, cyanide, azide and fluoride. Iodoacetate brought about the most rapid reduction in oxygen uptake, a concentration of only 3.3×10^{-5} M inhibiting respiration in four hours and a concentration of 10^{-3} M resulted in an inhibition of 73%. Cyanide and azide were also strong inhibitors, the former bringing about 79% inhibition in a concentration of 0.005 M and almost complete inhibition in a concentration of 0.01 M. Malonate and fluoride produced little inhibition except after some hours. The inhibitory action of azide and cyanide is held to indicate a cytochrome system, and the inhibitory action of iodoacetate a dehydrogenase, as concerned in the respiration of tomato roots. Succinic dehydrogenase would, however, appear to be absent, since malonate, which in animal tissues is regarded as a specific inhibitor of this, has no effect on the oxygen intake of tomato roots.

In an earlier section of this review the finding (23) has been noted that below a certain oxygen concentration there is a reduction of the respiration of the oat coleoptile to about half. It was found that treating the coleoptile with potassium cyanide in concentrations from N/3000 to N/100 reduced the rate of respiration to the same extent. It would therefore appear that cyanide and low oxygen concentration may inhibit the same system, and since the cytochrome-indophenol-oxidase system is sensitive to cyanide it is concluded that two oxidation systems are involved in the respiration of the oat coleoptile, one the cytochrome-indophenol-oxidase system and one insensitive to cyanide which might be that associated with the yellow ferment or flavin enzyme.

Brown and Goddard (17) found that the oxygen uptake by embryos removed from intact wheat grains was inhibited by hydrocyanic acid, sodium azide and by carbon monoxide, the inhibition of the last being light reversible. With 10^{-4} M sodium azide the oxygen uptake was reduced to about 25%, while 10^{-3} M hydrocyanic acid reduced it to about 9%. Since the inhibitors used inhibit the action of cytochrome oxidase, the conclusion is drawn that a large proportion of the respiration of the wheat embryo depends on cytochrome oxidase. A similar conclusion based on similar evidence was drawn in respect to barley seedlings (52, 56). Taylor (66) found also that approximately 70% of the respiratory activity of wheat seedlings and 50% of the respiratory activity of rice seedlings was inhibited by treatment with 10^{-4} M sodium azide, and concludes therefrom that at least part of the respiration of these plants is dependent on a cytochrome oxidase.

With the intention of obtaining information on the nature of the oxidising systems concerned in the respiration of barley, James and Hora (33) investigated the effect of cyanide on this process. They found that 0.002 M hydrocyanic acid brought about a depression of both oxygen absorption and carbon dioxide output of barley leaves, the effect being reversible. The depression of oxygen absorption was greater than that of carbon dioxide output. In nitrogen the rate of carbon dioxide output is about the same as in air until the output in the latter begins to rise, and addition of 0.002 M hydrocyanic does not affect the carbon dioxide evolution in nitrogen over this period. With prolonged exposure to an atmosphere of nitrogen, the rise in the respiration which would normally occur in starved leaves is very much less in an atmosphere of nitrogen, and if 0.002 M hydrocyanic acid is present as well the carbon output is still further reduced. At this stage of leaf starvation it is supposed that proteins are the principal respiratory substrate, and it would appear that lack of oxygen and presence of cyanide prevent proteolysis, a view supported by the facts that these conditions both delay or inhibit yellowing of the leaves and evolution of ammonia.

With a concentration of 0.02 M hydrocyanic acid respiration is completely and irreversibly inhibited.

From these results James and Hora conclude that an oxidation mechanism involved in respiration in barley is sensitive to cyanide, and that while glycolysis is not directly affected, cyanide inhibits proteolysis in nitrogen.

Following up this work, James and Cragg (31) examined the expressed juice of week-old etiolated barley shoots with a view to obtaining information on the enzymic mechanism of actively respiring barley. They conclude that polyphenol oxidase is absent from barley but that a redox system involving ascorbic acid is active in this plant. Thus when ascorbic acid is added to barley juice there is a considerable increase in oxygen intake which is completely inhibited by M/500 or M/1000 cyanide. No evidence could be obtained that the oxidation of ascorbic acid is due to peroxidase, polyphenol oxidase is absent, and the method of preparation of the extract precluded the presence of cytochrome. Hence James and Cragg conclude that barley extracts oxidise ascorbic acid by means of an ascorbic acid oxidase.

James and Cragg examined the oxygen uptake resulting when a number of substances were added to the juice along with the ascorbic acid. Of 13 organic compounds only three brought about a definite increase in oxygen absorption; these were glycollic acid, lactic acid and tartaric acid, lactic acid being particularly vigorous. It is therefore suggested that the reduction of the oxidised form of ascorbic acid to the reduced form is effected by a dehydrase with an α-hydroxy acid as hydrogen donator which is thereby reduced to an α-ketonic acid. Lactic acid, for example, would be reduced to pyruvic acid. The whole redox system can then be represented by

$$AH_2 + \tfrac{1}{2}O_2 \xrightarrow[\text{ascorbic oxidase}]{\text{by}} A + H_2O$$

$$A + R \cdot CHOH \cdot COOH \xrightarrow[\alpha\text{-hydroxy dehydrase}]{\text{by}} AH_2 + R \cdot CO \cdot COOH$$

It will be observed that in the three species, potato, carrot and barley, examined by Boswell, Marsh and Goddard, and James and Cragg, respectively, different systems are held responsible for the oxidation involved in respiration.

It has been suggested that the ascorbic acid system may be concerned in the production of phosphoglycerate from hexosediphosphate (32). On incubating barley sap for 24 hours at 30° C.

with hexosediphosphate in presence of thymol and sodium fluoride, the authors observed a loss of the hexosediphosphate and an increase in unhydrolysable phosphate which they conclude is phosphoglycerate. This effect was increased by addition of ascorbic acid. Also saps clarified by centrifuging, but not incubated, with added hexosediphosphate, absorbed little or no oxygen, but rapid absorption of oxygen took place on addition of ascorbic acid, an absorption in excess of that caused by ascorbic acid alone.

Since, when sap is incubated with hexosediphosphate in presence of sodium iodoacetate, an inhibitor of triosephosphate oxidation, the latter substance accumulates, and as hexosediphosphate is not readily oxidised whereas triosephosphate is readily oxidisable, it is thought that hexosediphosphate is first broken down to triosephosphate and that this is the substance actually dehydrogenated. The work of James, Heard and James appears to be the first suggesting a possible connexion between a well known oxidation system and glycolysis.

A contribution by Bennet-Clark and Bexon (11) sheds light on the mechanism of plant respiration. They record first that a considerable increase in the respiration of thin slices of red beet root takes place when these are immersed in the expressed sap of the beet root. When undiluted sap is used the increase may be as much as 140% or more, and even sap diluted with water to 0.13% will bring about a 20% increase in respiration. This rise in respiration rate is accompanied by an increase in the respiratory quotient to a value considerably above its normal value of about unity, for the rise of carbon dioxide output is not accompanied by a corresponding absorption of oxygen. The greatest rate is reached after about half an hour and with only a slight fall is maintained for many hours. Fractionation of the sap and immersion of tissue in the various fractions indicated that organic acids, particularly malic and citric acids or their salts, were the constituents of the sap responsible for the observed effect, and this was confirmed by experiments in which slices of tissue were immersed in solutions of the sodium salts of malic, citric and succinic acids. The effects of these substances are very similar in every respect to those of expressed sap.

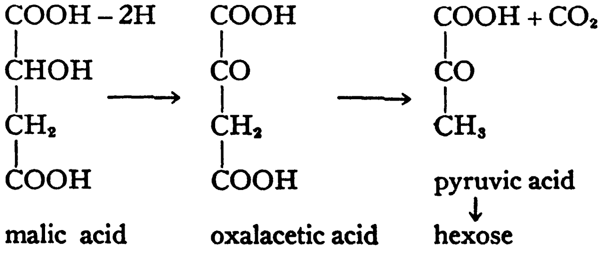
Bennet-Clark and Bexon next record the results of an examination of the effects of plasmolysis of the cells of beet root tissue on their respiration. These effects are quite distinct from those produced by applying cell sap externally to the cells. Along with plasmolysis there is at first a rapid rise in respiration followed by a fall to a steady value at a level somewhat below the original pre-plasmolysis value.

As regards the effect of sap, Bennet-Clark and Bexon point out two conditions, either of which could produce the observed results. The first of these is that the external plasmatic membrane should be more permeable than the inner plasmatic membrane, the second is that the respiratory enzyme systems should be situated on the external surface of the protoplast. Either would explain why malate, citrate or any other substance bringing about a rise in respiration rate should be more effective applied externally to the cell than in the vacuole.

Bennet-Clark and Bexon compared the loss of malate, citrate and succinate from tissue slices supplied with these, with the carbon dioxide evolved in excess of the normal amount given out by tissue respiring in water. They found that about one molecule of acid is lost for every molecule of carbon dioxide evolved. Since these acids contain four carbon atoms in each molecule, this means that only a quarter of the carbon of these acids is lost as carbon dioxide. Bennet-Clark and Bexon consider it possible that the rest of the carbon appears in hexose so that the fate of a molecule of malic acid may be expressed as

$$C_4H_6O_5 = \tfrac{1}{2}\, C_6H_{12}O_6 + CO_2.$$

An enzyme (malicdehydrogenase) in animals is known which will effect the oxidation of malic acid to oxalacetic acid, and the interaction of pyruvic acid and carbon dioxide to give oxalacetic acid or a derivative of it is known to take place in certain animal cells and bacteria, while the production of carbohydrate from pyruvic or lactic acid was demonstrated a quarter of a century ago in liver and some types of muscle by Meyerhof. The stages in the formation of hexose from malic acid might thus be represented:



$$\begin{array}{lclcl}
\mathrm{COOH-2H} & & \mathrm{COOH} & & \mathrm{COOH+CO_2} \\
| & & | & & | \\
\mathrm{CHOH} & & \mathrm{CO} & & \mathrm{CO} \\
| & \longrightarrow & | & \longrightarrow & | \\
\mathrm{CH_2} & & \mathrm{CH_2} & & \mathrm{CH_3} \\
| & & | & & \\
\mathrm{COOH} & & \mathrm{COOH} & & \text{pyruvic acid} \\
 & & & & \downarrow \\
\text{malic acid} & & \text{oxalacetic acid} & & \text{hexose}
\end{array}$$

Supporting evidence for the view that malic acid is not merely oxidised to carbon dioxide and water is provided by the respiratory quotient of tissue supplied with malate, for when the latter is provided in a concentration of about 0.05 N, about equal to that of malate in the sap, the respiratory quotient lies between 1.5 and 2.3 and is thus higher than a quotient provided exclusively by the oxidation of malate (1.33) and still higher than one provided by a combination of malate and carbohydrate combustion. It would, however, be given by a decarboxylation of each molecule of malic acid to provide one molecule of carbon dioxide and half a molecule of hexose. The resynthesis of carbohydrate from malate is regarded by Bennet-Clark and Bexon as comparable with that oxidative anabolism which was first demonstrated in apples by Blackman and Parija.

The effects of plasmolysis on respiration are explained as follows. In the unplasmolysed cells the malate and citrate in the vacuole diffuse across the inner plasmatic membrane to reach the respiratory enzyme system, and the slow rate of the diffusion across this membrane controls the rate of respiration. Plasmolysis of beet root cells is at first concave, and the area of the boundary between vacuole and protoplasm actually increases; later the protoplast contracts completely away from the cell wall, and the area of the internal plasmatic membrane then decreases. Assuming the amount of material in the membrane remains constant, the thickness of the membrane must first diminish and then increase.

Now the rate at which malate and citrate reaches the respiratory enzyme centres will be proportional to the concentration gradient and the area of the membrane, and inversely proportional to its thickness. In the first stage of plasmolysis, while it may be supposed there is little change in the concentration gradient, the increase in area and decrease in thickness of the plasmatic membrane will both condition an increase in the rate of diffusion of malate and citrate to the respiratory enzyme centres and so to an increase in respiration. But as concave plasmolysis gives place to contraction of the protoplast away from the cell wall, the inner plasmatic membrane decreases in area and increases in thickness and both these changes will lead to a decrease in the rate of diffusion of the malate from vacuole to enzyme systems and so to a decrease in respiration rate. On the other hand, the reduction in the volume of the cell on plasmolysis means an increase in the concentration gradient, an increase in the rate of diffusion and increase in the rate of respiration. Bennet-Clark and Bexon, in evaluating these various factors, estimate that a reduction in the volume of the cell to one-half would bring about a reduction of 21% in the rate of diffusion of malate and citrate from the vacuole. Actually they observed initial increases in respiration on plasmolysis of about 40% or more followed by a fall to a steady value about 25% below the pre-plasmolysis level, in tissue plasmolysed with potassium chloride, calcium chloride or glucose in concentrations sufficient to reduce the volume of the protoplast to about half its initial volume.

ANAEROBIC RESPIRATION, THE PASTEUR EFFECT AND OXIDATIVE ANABOLISM

It has been mentioned earlier in this review that the Pasteur effect refers to the inhibition of sugar breakdown owing to the presence of oxygen. Since in aerobic respiration the complete breakdown of a molecule of hexose to carbon dioxide and water should result in the production of six molecules of carbon dioxide, while the breakdown of a molecule of hexose in anaerobic respiration fermentation to carbon dioxide and ethyl alcohol gives only two molecules of carbon dioxide, it may be concluded that the Pasteur effect is operative when the rate of evolution of carbon dioxide in air is less than three times the rate of evolution of this gas in nitrogen or hydrogen, that is, when the ratio of respiration (as measured by carbon dioxide output) in nitrogen to respiration in air is greater than 0.33.

During recent years a number of additions have been made to the list of tissues in which the Pasteur effect has been shown to occur. Choudhury (20) observed it in red beet root and artichoke tuber in which the ratio of respiration in nitrogen to respiration in air is about 0.75, and particularly in carrot where it was found to average about 1.36. Both Marsh and Goddard (55) and Turner (74) found a similar very considerable Pasteur effect in thin slices of carrot root. The former record that on transference from air to nitrogen the output of carbon dioxide from carrot slices increases by about 15%. Turner found the effect of transferring thin slices of carrot from air to nitrogen depended on the rate at which the tissue was respiring at the time of transference.

With disks supplied with air Turner recorded a general drift downwards in the respiration rate with time to a low level which he called the basal rate. On transference to nitrogen of tissue disks respiring at this low rate, the rate of carbon dioxide output in nitrogen was equal to, or slightly higher than, the final rate in air, but where the rate in air was high, transference to nitrogen brought about a lowering in carbon dioxide output. The rate of carbon dioxide output in nitrogen increased with time, a result agreeing with Choudhury's observations on uncut carrot roots.

No evidence for the existence of a Pasteur effect during the germination of seeds of species in which carbohydrate constituted the principal reserve could be found by Leach (45). He found the ratios of the respiratory rate in nitrogen to the respiration rate in air in *Lathyrus odoratus, Fagopyrum esculentum* and *Zea mais* were respectively, about 0.22, 0.35 and 0.25. Taylor (66), however, found quite a considerable output of carbon dioxide by wheat seedlings in nitrogen and a very big output by rice seedlings, the ratio of carbon dioxide output in nitrogen to that in air for the two species of seedlings being, respectively, 0.56 and 1.53. The values of the ratio found by Choudhury for whole potato tubers were about 0.40, a value so little above 0.33 that it cannot be regarded as evidence of the Pasteur reaction; moreover, with this tissue the interpretation of results are rendered difficult on account of the fact that no or little ethyl alcohol is recognisable as a product of anaerobic respiration.

It is well known that Blackman explained carbon dioxide evolution in nitrogen at a rate above one-third that in air not as due to a suppression of glycolysis by oxygen, but as due to oxidative anabolism by which intermediate products of the breakdown of hexose were built back into the system. Marsh and Goddard discuss their results in relation to the theory of oxidative anabolism and also to Lipman's theory, that would explain the lowered output of carbon dioxide in air as due to oxygen in respiration acting through a carrier with a fermentation enzyme, which is thereby inactivated so that the rate of glycolysis is lowered. While they hold that their results are consistent with, but do not afford proof of, the theory of oxidative anabolism, their results can be regarded as consistent with Lipman's theory only if the respiratory poisons not only inhibit respiration but also remove the inhibition of the oxidation of the fermentation enzyme system. This would be the case if the supposed inhibition of fermentation by oxygen is catalysed by cytochrome oxidase, an enzyme which, as we have seen, they consider plays a part in the normal respiration of the carrot.

In Turner's experiments the rate of carbon dioxide output in nitrogen was more than one-third that in air, so that Turner considers there is evidence here for oxidative anabolism as hypothesized by Blackman, but the found ratios of oxidative anabolism to aerobic respiration, in terms of carbon atoms involved, are very variable. The results can, however, be explained by supposing that when the tissue is cut the rate of glycolysis is increased two or three times and the respiration rate up to five times, but that the rate of oxidative anabolism remains constant. Thus while in uncut roots the ratio of oxidative anabolism to aerobic respiration is about 3, in wounded tissue it may fall to as low a value as 0.5.

Boswell and Whiting (15) found a temporary increase in the rate of output of carbon dioxide by potato tubers kept at 29° C., but conclude that this increase results from the release of carbon dioxide not from cell respiration, but from carbon dioxide chemically bound in the tissues as bicarbonates or with amino-acids and proteins. Under anaerobic conditions, according to Boswell and Whiting, the capacity of potato tuber tissue to bind carbon dioxide is less than under aerobic conditions, for the carbon dioxide evolved on treating tissue with 10% sulphuric acid, assumed to be this bound carbon dioxide, is less under anaerobic than under aerobic conditions. Consequently, on transferring the tissue from air to nitrogen the tissue can retain less bound carbon dioxide and so some of this is released. Because the increased amount of carbon dioxide given off as a result of transferring the tubers from air to nitrogen is of the same order as the difference in the bound carbon dioxide released from slices of potato tissue treated with acid under aerobic and anaerobic conditions, Boswell and Whiting conclude that the excess carbon dioxide given off on transferring tubers to nitrogen is wholly due to a release of previously bound carbon dioxide, and that therefore there is no need to assume the existence of oxidative anabolism. It should, however, be noted that Blackman's theory of oxidative anabolism requires, not that the rate of anaerobic respiration should exceed that of aerobic respiration, but that it should exceed only one-third of the aerobic respiration rate. The values found by Boswell and Whiting rather suggest that on transference of potato tubers from air to nitrogen there is still an excess of carbon dioxide output over one-third the previous aerobic respiration rate after allowing for the release of the bound carbon dioxide after transference to anaerobic conditions. However, the difficulty of interpreting results with potato tuber tissue has already been mentioned.

In earlier work Thomas had shown that ethyl alcohol and acetaldehyde accumulate in apples exposed to the vapour of hydrocyanic acid, the phenomenon called by him HCN-zymasis. He and Fidler (69, 70) have now made a detailed study of this. They found that healthy apples absorb hydrocyanic acid from their vapour, the rate of accumulation of the substance being determined by its external concentration up to a certain value of the latter. When the rate of cyanide accumulation exceeded a certain value aldehyde and alcohol accumulated in the tissue, the rate of their accumulation being dependent on the rate of cyanide accumulation, while the reduction of acetaldehyde to ethyl alcohol appeared to be retarded. The zymasis induced by cyanide can be explained on the view that oxidising enzymes involved in the normal respiration mechanism are poisoned by cyanide. This inhibition of enzyme activity appears to be reversible, since zymasis stops when apples containing low concentrations of cyanide are returned to air.

By measuring the alcohol + aldehyde produced, in addition to their carbon dioxide output and oxygen absorption, by apples in conditions inducing HCN-zymasis and also by apples in pure air, Thomas and Fidler have attempted to analyse the total carbon dioxide output of the former into the carbon dioxide of normal respiration and the carbon dioxide of zymasis. They find that the accumulation of alcohol and aldehyde may be accompanied by a decrease in the rate of oxygen absorption and hence of normal respiration. The rate of total carbon dioxide output may, however, be increased. It is concluded from this that hydrocyanic acid, by inhibiting the activity of the oxidation enzymes, increases the rate of breakdown of the hexose substrate. This means that the so-called Pasteur effect operates in the normal respiration of apples, and from their experimental data Thomas and Fidler conclude that the aerobic respiration of one molecule of hexose preserves about two molecules from breakdown.

CONCLUSION

From this review covering research published during the last ten years it will be seen that this period has been a very active one as far as work on respiration is concerned. Particularly has attention been directed to the mechanism of the respiratory processes, and it may be fairly claimed that real advances in our knowledge of this complex question have been made. Considerable interest has been shown in the connexion between the various oxidising systems known to exist in plant cells and repiration, so that whereas in 1935 practically nothing was known of the part played by these systems, to-day a very considerable mass of evidence has accumulated indicating the relationship between various oxidising systems and the respiratory activity of plants of different species. If the conclusions of the different workers are somewhat conflicting, this is not surprising in a line of work which has received attention so comparatively recently. In a field which is attracting so much attention we may expect that before long many of the apparent divergences will be explained.

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THE BOTANICAL REVIEW

VOL. XII APRIL, 1946 No. 4

AEROBIOLOGY IN RELATION TO PLANT DISEASE[1]

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It can be argued that the greatest harm that man has done himself in crop production has been by transporting plant pathogens from region to region and continent to continent, often to land areas to which neither wind nor insects nor any other agent of dissemination besides man would have carried them initially. Man was clearly responsible for intercontinental distribution of the pathogens causing late blight of potatoes, powdery and downy mildews of grapes, powdery mildew of gooseberries, downy mildew of hops, chestnut blight, white-pine blister-rust, citrus canker, the so-called Dutch elm disease, bacterial blight of pomaceous fruits, many plant viruses, and scores of others. Possibly wind, insects or other agents apart from man himself might eventually have carried these pathogens several thousand miles from their original homes, but the fact is that the job apparently was not done until man did it. In many cases, it is true, wind and insects took over where man left off, and greatly expanded the area of infestation from the small man-made beginnings. It is the wind, however, which is on trial in this paper, not man and insects, but perspective on the entire problem would be distorted if the importance of man, insects, other animals and water were ignored, for many destructive plant pathogens are not well adapted to effective dissemination by wind alone and therefore must depend on other agents of distribution.

The little information that is available regarding wind dissemination of phytopathogenic bacteria is to the effect that some are carried at least short distances (6, 33, 38, 53, 58, 99); and information regarding slime molds, if they are considered independently, is very meager. The seeds of phanerogamic parasites are not carried principally by wind; and there is no conclusive evidence regarding wind dissemination of free particles of plant viruses in nature, although the wind certainly is an aid to the migration of certain viruliferous insects and therefore an important factor in the spread of some virus diseases (15, 21, 40, 63, 67, 108, 143). Further investigations are needed on bacteria and viruses. So far as is now known, however, wind is the most dangerous agent in the dissemination of certain plant pathogenic fungi.

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[1] Paper No. 527, Miscellaneous Journal Series, Minnesota Agricultural Experiment Station.

[2] The authors wish to express appreciation to J. J. Christensen and Helen Hart for critical reading of the entire manuscript; to E. G. Sharvelle, M. B. Moore and Carl J. Eide for reading of sections; to D. G. Fletcher for assistance in obtaining data on stem rusts; and to Laura M. Hamilton for assistance in preparation of the manuscript, especially those sections that deal with the epidemiology of cereal rusts.

That spores of fungi are disseminated by air currents has been known almost as long as the spores themselves. Micheli in 1729 published the results of epoch-making investigations on the reproduction of fungi by means of spores and showed that clouds of them might be liberated into the air (81). Prevost in 1807 not only showed that bunt of wheat was caused by a fungus but also suggested that the spores might sometimes be disseminated by the wind at threshing time (92). After DeBary furnished generally accepted evidence in 1853 that fungi could cause plant disease (28), there gradually developed the realization that many fungus pathogens of plants were disseminated, at least locally, by wind. But there still was no conclusive evidence for long-distance dissemination, although Klebahn (61) and others postulated it because of the epidemic occurrence of certain rust diseases at considerable distances from known sources of inoculum. The results of numerous observations and experiments, however, have made it clear that wind is probably the commonest agent of dissemination of phytopathogenic fungi (14, 20, 22, 24, 38, 41, 58, 82, 117, 118).

Naturally, the evidence for local dissemination is more extensive than that for long-distance dissemination; but there now is proof that regional epidemics of some diseases, notably cereal rusts, result from rapid and widespread dissemination of spores by air currents. The possibility of effective dissemination of spores across great barriers such as wide oceans, high mountain ranges and extensive deserts remains to be investigated thoroughly.

ADAPTATION OF FUNGI TO AERIAL DISSEMINATION

Many fungus pathogens are remarkably well adapted to wind dissemination, for they produce almost countless numbers of small and very light spores that are shot forcibly into the air or are separated easily from each other and are sufficiently hardy to withstand the rigors of a long air journey. The quantity produced by many fungi and the marvelously effective devices for efficient utilization of space in their production are as noteworthy as they are important. This prodigal production of spores has great survival value for those parasitic fungi whose spores are disseminated primarily by the wind, because the wastage of spores often is tremendous. Their "soil" is host plants, and in some cases particular organs of particular varieties of particular kinds of crop plants. The dissemination is passive; it is subject to the vagaries of wind and weather. There is no purposeful migration as is possible with many insect pests. Nor is there virtually unlimited "soil" as is available to seed plants. On the contrary, appropriate host plants in the proper stage and condition for infection often are limited in amount, especially in regions where agriculture is diversified. Consequently the plant pathogen must overcome this handicap by producing large numbers of spores or other propagative bodies. Although bits of mycelium and sclerotia may be carried to a limited extent by the wind, spores predominate so greatly as organs of multiplication that consideration of air dissemination may be restricted to them.

QUANTITIES OF SPORES AND DEVICES THAT FACILITATE THEIR PRODUCTION

The number of spores produced by various plant pathogenic fungi has been determined, the fruit bodies of some wood-rotting species producing especially large quantities. A single fruit body of *Daedalia confragosa* (Bolt.) Fr., for instance, two square inches in size, produces about 682 million spores; a large fruit body of *Polyporus squamosus* (Huds.) Fr., 50 billion; and a large fruit body of *Fomes applanatus* (Pers.) Gill. produces 5,460 billion, discharged at the rate of 30 billion a day for six months (8). Even a relatively small fruit body of an Ascomycete such as *Sclerotinia sclerotiorum* (Lib.) Fuck. produces about 31 million ascospores (138). *Peronospora schleideni* Ung., the organism causing downy mildew of onions, produces 140 thousand conidia per square inch of leaf surface (148); *Sphaerotheca humuli* (D.C.) Burr, powdery mildew of hops, produces 2,280 thousand conidia per square inch of leaf surface (4); and the apple scab fungus, *Venturia inequalis* (Cke.) Aderh., discharges about 5,630 ascospores from one square centimeter of leaf surface in 45 minutes, or 8,107,200,000 under one tree in an orchard (142). Small wonder that such plant pathogenic fungi can be disseminated widely in a short time!

Some of the smuts and rusts also produce almost countless numbers of spores. A single wheat kernel converted into smut by *Tilletia tritici* (Bjerk.) Wint. contains from six to 12 million spores, and there would be about 5,000 billion on an acre of wheat if only 1% of the heads were infected (7, 20, 46). *Ustilago zeae* (Beck.) Ung., the ordinary corn smut fungus, produces about six billion spores in a cubic inch of smut-gall tissue, and even a moderate infection of 10% in a cornfield would result in the production of 50,000 billion spores per acre (20). Moreover, these spores on germination may produce sporidia that are capable of indefinite multiplication by budding in water, in the corn plant, on the soil or near manure piles. Thus an incalculable number of sporidia may be produced saprophytically. Among the rust fungi, a single cedar gall of *Gymnosporangium juniperi-virginianae* Schw. is said to produce nearly 7,500 million basidiospores (65). There are more than 60 million aeciospores of *Puccinia graminis* Pers. at one time on a fairly heavily rusted six-foot bush of *Berberis vulgaris* L., and several crops of spores may be produced on a single bush during spring and early summer. Each of these spores is potentially able to cause the production of from 50 thousand to 400 thousand urediospores on grains or grasses within a week (124). On an acre of slightly rusted wheat there may be 10,000 billion urediospores, and several times that number on an acre of heavily rusted grain (20). Indeed, clouds of "rust dust" can often be seen rising into the air when heavily rusted wheat is being harvested; and machines, men and the soil often are red with countless numbers of spores.

Many plant pathogenic fungi are thus known to produce spores in almost incalculable numbers, and the results may be catastrophic when conditions are favorable for widespread dissemination, germination and infection of susceptible crop plants. Figuratively and literally, infection may spread with the speed of the wind, for the wind itself often carries billions upon billions of agents of destruction for crop plants. It is fortunate that the mortality rate among spores is high; were it not, plant disease epidemics would be even more terrible than they often are.

Up to a certain point the fungi seem to have provided all necessary safeguards to success, and they have not succeeded in becoming so inordinately prolific without many ingenious biological inventions, among which are extremely efficient structures and processes for producing spores and insuring their liberation.

It is beyond the scope of this summary to detail the astonishing achievements of the fungi in producing myriads of spores on or in relatively small structures. Zopf, Buller, and others have done this (7, 8, 9, 10, 11, 12, 151). Nevertheless, the resourcefulness of many parasitic fungi in overcoming the handicap of limited plant surfaces on which to produce their spores is basic to their success as living organisms that must depend on the wind for their distribution. The production of many small spores in sporangia instead of one or a few larger ones is, of course, one of the simplest methods of attaining the desired end. The various methods of branching, the production of spores in chains, and the aggregation of sporophores into compact palisades enable fungi to produce large numbers of spores on a relatively small surface. Many fungi have developed the architectural principle of vertical extension, which enables them to utilize the air in two ways: space for multiplication and motive power for migration. And somehow, somewhere, some of them acquired genes for the production of fruit bodies that in many respects are the architectural marvels of the plant world. The aggregation of asci in the various kinds of fruit-bodies of the Ascomycetes, with the vertical rows of ascospores held in place until they are ripe and ready to be shot out into the air, challenges the imagination as to the mechanisms of action of the genes. These fruit bodies are wonderfully effective, furthermore, in enabling the fungi to hedge against future hazards, for they produce so many spores that there is a wide margin of safety against losses.

Some of the Basidiomycetes are even more remarkable. The Agaricales, for instance, probably have the most efficient spore factories of any fungi. Indeed, it is doubtful that any other plants have attained such perfection in production of propagative bodies. Instead of producing spores on a flat hymenial surface, they produce them on basidia standing at right angles to accurately spaced gills, teeth or pores. The fruit body of *Fomes applanatus* (Pers.) Gill., a bracket fungus, produces spores on basidia lining the inner walls of tubes averaging 10 to 20 millimeters in length and only 0.17 millimeter in width. There are about 2,000 of these tubes per square centimeter of surface, and a new layer may be produced each year for several years. The spore-bearing surface is about 148 times as great as that of a flat surface. It has been calculated that one medium-sized fruit body has about two million tubes, each liberating 15 thousand spores a day, a total of 30 billion spores every 24 hours. This may go on daily for six months (8). Moreover, most of these spores normally reach the open air, where air movements can carry them away. And it is not by mere chance that the spores are thus liberated. On the contrary, the genes of many fungi seem to have provided for every contingency, up to a certain point.

SPORE WASTAGE

As concerns their aerobiological relationships, fungi are confronted with the necessity of producing enormous numbers of spores because of the slight chances that the spores will be carried to appropriate infection courts at appropriate times. As the fungi under consideration are parasitic on plants, the surface available for spore production is limited. This handicap has been overcome by producing extremely efficient but complex fruit bodies. The very fact that so large a number of spores is produced in so small a space, however, poses the problem of wastage even before the spores are liberated from a fruit body in which crowding jeopardizes liberation. If a fungus could worry, it would have plenty to worry about. *Fomes applanatus,* for example, produces spores in tubes so long in proportion to their diameter that even a slight deviation from the perpendicular would prevent a considerable proportion of the spores from falling the length of the tube and getting into the open air, even if most of the spores were produced near the lower end of the constantly growing tubes. Likewise, when spores are produced in vertical rows, as in pustules, or aecial cups, or asci, their dispersion must depend on their separation from each other and their liberation from the receptacles within which they are formed. This dispersion is one of the prerequisites to biological success, from the viewpoint of the fungus, and to the epidemic development of plant diseases, from the standpoint of man.

LIBERATION OF SPORES

Some fungi have not developed any special method for liberation of spores from the structures on or in which they are formed. But even among the least specialized the method of attachment of the spore to the sporophore is such as to permit easy separation by even very light air movements, such as those caused by the "wash" of air from a fly's wings. Moreover, many fungi that produce spores in chains provide sterile cells, sometimes modified into special disjunctors, that facilitate easy separation. And the forcible abjection of sporangia or conidia in many of the Phycomycetes and the almost universal abjection of basidiospores in the Basidiomycetes not only is an interesting and complex biological process but is probably universally an aid to wind dissemination. Similarly, the forcible ejection of ascospores from asci is a first step in permitting wide dissemination. The mechanisms of puffing in the Discomycetes and the ejection of spores from successive asci in perithecia having only a small ostiole have been adequately described, and further consideration is beyond the scope of the present paper. Likewise, the way in which aeciospores of certain rust fungi are shot out into the air has been studied and described. Spore liberation is facilitated still further in many fungi by a series of responses to various stimuli that place the fruit body and its spore-bearing surface in the most favorable position for discharging the largest possible number of spores into the air. From the general biological standpoint, spore liberation is extremely important, but our present concern is principally with the spores after they get into the air.[3]

Although many fungi produce almost incomprehensibly large numbers of spores in relatively small space and then liberate them with remarkable precision, they seem to have invented no special structures, such as the wings or parachutes of some higher plants, to aid the spores themselves in their air journeys. It is true that echinulations and reticulations of the spore wall may increase their buoyancy somewhat, but there seems to be no relation between the presence or absence of such modifications of the wall and the degree to which the spores are disseminated by the wind. Indeed, the spore walls of many of the conspicuously wind-disseminated species are smooth or nearly so. In any case, because of the minuteness of spores such modifications are not necessary, and it is difficult to see how they could be anything but a handicap to the processes of spore production and liberation, as they have evolved. Thus the fungi have developed remarkable efficiency in the production of extremely large numbers of very buoyant spores, and seem to have got the utmost in survival value out of the energy and ingenuity expended.

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[3] **The statements in this paragraph are so generally known that specific references for all statements would be superfluous. The following references contain the best summaries: 7, 8, 9, 10, 11, 12, 52, 150.**

SIZE OF SPORES

The spores of phytopathogenic fungi range in size from about five microns to 125, rarely up to 300. The conidia or zoosporangia of Phycomycetes, mostly disseminated by wind, are about 25 × 15 microns; ascospores of many of the common Ascomycetes are generally within a range of 10–25 × 5–15 microns. Urediospores of rusts, usually rounded, elliptical or cylindrical in shape, range, in general, from about 15 to 35 microns in one or the other diameter. Smut spores are mostly somewhat smaller than those of rusts, and basidiospores are still smaller, mostly about five to eight by three to five microns. The largest spores are produced by some of the Fungi Imperfecti, notably the genera *Helminthosporium, Alternaria* and *Macrosporium*. But even the really large spores do not often exceed 150 microns in length. Naturally, such small objects are well suited to aerial distribution, as they fall very slowly, even in still air, and can therefore be carried upward by convection currents, and then long distances by winds.

ALTITUDES REACHED BY SPORES, RATE OF FALL, AND THEORETICAL DISPERSAL DISTANCE

Although fungus spores are generally more abundant in the air near the earth than at higher altitudes, several investigators have encountered clouds of them or numerous bacteria one to several thousand feet above the earth, seemingly borne along by the wind as more or less discrete sharply bounded units (13, 26, 27, 76, 77, 89, 93, 94, 95, 98, 123, 149). Urediospores of *Puccinia graminis tritici* (Pers.) Eriks. and Henn., for instance, have been caught as high as 14,000 feet above infected grain fields (89), living spores of various fungi were caught from aeroplanes above the Caribbean Sea 600 miles from their nearest possible source, and living spores of several common molds were caught in a spore trap released from the balloon Explorer II at 72,500 feet and set to close at 36,000 feet (74, 75).

The approximate rate of vertical fall of spores of different shapes and sizes through still air has been determined in the laboratory under various conditions, and ranges from about 0.5 millimeter per second for the smallest and lightest spores such as the basidiospores of *Collybia dryophila* Fr. and conidia of *Hormodendrum resinae* Lindau[4] (*ca.* 3 × 5 μ in size) to 20 millimeters per second in spores of *Helminthosporium sativum* P. K. and B. (20 × 75 microns) (7, 20, 68, 69, 139). In most of these measurements on rate of fall the relative humidity of the air through which the spores fell was low, and it is known that the rate of fall in dry air may be only one half or one third that in humid air, presumably because in humid air the spores imbibe water and become much heavier (7). This may be an important factor in the initiation of epidemics from air-borne spores, since the spores are likely to settle out of the air more rapidly under humid conditions that favor infection of the host plants.

From the data obtained in laboratory experiments it has been calculated that *Alternaria* spores which had attained a height of one mile, falling at the rate of three millimeters per second could be carried at least 2,900 miles by a 20 mile-an-hour wind; and urediospores of *Puccinia graminis tritici,* falling at the rate of 12 millimeters a second from a height of one mile could be borne at least 740 miles by a 20 mile-an-hour wind (20). Since spores of these and many other plant pathogenic fungi have been caught in large numbers at altitudes greater than a mile, and since winds of greater velocity than 20 miles an hour commonly travel for hundreds or even thousands of miles across the continents and intercontinental oceans, these estimates of dispersal distances probably are conservative. It is possible that horizontal winds alone are sufficient to maintain spores in steady flight, even as the water flowing in a river will carry comparatively heavy sediment for hundreds of miles before it is deposited where the speed of the current is reduced. Preliminary experiments have been made in our laboratory in an attempt to determine whether horizontal air movement of a few feet per minute is sufficient to maintain spores in steady flight, but insufficient data have been gathered to justify inclusion here. Data of this kind would be of obvious value in epidemiological work. Numerous observations, however, indicate that many of the smaller fungus spores are more at the mercy of air currents than of gravity; they do not really fall to earth or settle out of the air but rather are carried down by downward air currents or by various forms of precipitation. There seems to be no reason to suppose that under favorable conditions spores may not be carried across continents and oceans. One important task of future aerobiological research will involve the charting of aerial trade routes over which a tremendous tonnage of contraband biological freight may be carried.

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[4] Determinations on *Hormodendrum* and *Helminthosporium* made by C. M. Christensen.

Theoretically, then, spores can be carried almost unlimited distances, and evidence supports theory. The numbers of spores that are often found on glass slides exposed from airplanes at altitudes up to 10,000 feet is eloquent testimony of the efficacy of the air as a carrying agent. The further fact that spores often are caught long distances from any known or possible source of production is still more conclusive evidence. But the most important question remains to be answered: How far are spores carried effectively; what is the relation of air-borne inoculum to the development of plant diseases?

LOCAL DISSEMINATION

Effective dissemination of inoculum depends on many factors other than mere dissemination of spores. Spores obviously must be viable when they are deposited on host plants, the host plant must be in the proper stage of development and in proper physiologic condition to permit infection, and either the wind-borne inoculum must be very abundant or conditions following infection must be favorable for rapid multiplication of the pathogen before a disease can become epidemic. The practical test of effective dissemination is effective dissemination itself. There is abundant evidence that effective local dissemination by wind is very common (58), but it would be quite impossible to review all evidence in detail; therefore certain cases have been selected to illustrate certain principles of plant disease development and control:

a. Venturia inequalis, causing apple scab, furnishes a good example of the practical importance of knowing intimate details regarding air-borne spores. The fungus survives the winter, in most regions, principally on diseased leaves. On these fallen leaves the ascus-containing perithecia are formed during the dormant season. The ascospores are discharged forcibly into the air during periods of wet weather in the spring and early summer, not all at one time, but at several periods. The time and number of ascospore discharge periods varies with locality and season. At Madison, Wisconsin, the discharge periods in 1921 were from April 23 to June 12, and at Sturgeon Bay in the same State from May 20 to June 30 (57). At Madison the time of ascospore maturity ranged from March 22 to April 26 over a period of six years, but always preceded blossoming of the apples (60). In Virginia there were 16 discharge periods between April 18 and June 12 in 1922, and 13 periods between April 28 and July 30 in 1923 (106). The principal factor in ascospore discharge is rainfall, temperature being relatively unimportant. Spores, therefore, are likely to be present in the air in an orchard early in the season. It has been calculated that about eight billion ascospores may be discharged under a single tree in 45 minutes, and considerable numbers have been caught in orchard air; hence initial infection is well provided for (36). The conidial stage then multiplies more or less rapidly, depending on moisture and temperature. Just how far the ascospores are carried is not known, but they are rather resistant to unfavorable conditions and probably can survive journeys of considerable length. Initiation of scab infection is therefore rather easy. Inoculum usually is present early in the season in those regions where conditions favor development of the disease; the ascospores are shot out from one ascus after another in such a way as to insure their reaching the air; and the rains required for their discharge are favorable for infection also. The spores therefore usually are not subjected to unfavorable conditions, as they are liberated only during wet weather, in contrast to many spores that are disseminated principally during dry weather, such as those of some rust and smut fungi.

Practical applications are made of these facts in controlling apple scab. In many important apple-growing regions the use of fungicidal sprays in spring is timed on ascospore discharge, the attempt being to get the spray on the plants just before the ascospores get there and cause infection. Moreover, disease outbreaks are even nipped in the bud by using eradicant sprays directed against the fungus on apple leaves on the ground in orchards (59).

In apple scab, therefore, knowledge regarding the aerobiological phases of the etiology of the disease has aided greatly in more effective control (60).

b. Phytophthora infestans (Mont.) de B., which causes late blight of potatoes, is a typically epidemic pathogen that can become widely prevalent and devastatingly destructive in a short time. All circumstantial evidence has pointed to wind dissemination of the so-called conidia or zoosporangia, the only propagative bodies known to be produced commonly. There have been various explanations for the source of inoculum in the spring, the most plausible being that the pathogen might overwinter on infected tubers in the soil in some regions or be introduced into fields with infected tubers at planting time (1, 29, 55, 80, 83). Recently it has been shown that a common source of inoculum in northern United States is refuse piles of potatoes. Studies in Maine (5) furnished evidence that the fungus often sporulates freely on sprouts from infected tubers in refuse piles in the spring or early summer and that the spores are then blown to nearby fields. What appeared to be conidia of the fungus were caught on slides 1,000 feet from the refuse piles, and the disease was observed to spread 600 feet from such piles, always in a northwesterly direction, because winds are from the southeast during periods of rain in that region. As the severity of infection decreased rather rapidly with increasing distance from the refuse piles, the evidence seems conclusive as far as it goes. These observations in Maine have been at least partially confirmed by similar observations in Minnesota[5] and elsewhere. Circumstantial evidence is strong that subsequent spread results from infection by wind-borne spores formed on plants in the initial infection centers. But it is not known how far they are carried. There are some indications that the pathogen may move from southeastern United States northward to the northeastern states when wind direction is favorable during periods of rainy weather in early spring. There are indications also that the disease may spread westward in the Lake States during periods of cool wet weather when the winds are from the east-northeast. The difficulty in obtaining more precise information is due to the sporadic occurrence of the disease in some regions and the difficulty of identifying the spores with certainty when caught on vaselined slides. Nevertheless, observations in Minnesota support the hypothesis that wind dissemination plays an important part in the development of widespread regional epidemics, as the disease sometimes spreads westward across the state in a season after several seasons during which no blight can be found. The problem is an important one, especially in those regions where the disease becomes destructive only occasionally and where more information regarding spore dissemination could lead to more effective and economical spraying procedures for control of the disease.

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[5] Unpublished results of C. J. Eide, R. C. Rose and W. D. Thomas.

c. Sclerospora sp. Local dissemination of downy mildews of maize in the Philippine Islands has been proved and long-distance dissemination has been suspected (147). Two pathogens cause the disease: *Sclerospora spontanea* Weston and *S. philippinensis* Weston. The organs of multiplication are conidia which are produced only at night when the plants are wet with dew. So rapid is their formation that from a single maize plant in a single night almost six billion may be liberated. They are forcibly thrown off from the conidiophores and are carried away by almost imperceptible air movements. During an apparently perfectly calm night, for instance, conidia were caught on agar in Petri dishes 80 feet from infected plants. By tracing infection following strong winds blowing from infested areas to areas free from infection, evidence was obtained that the conidia could be carried in viable condition for at least several miles. "Spread of these diseases in the Philippines ... is chiefly local and discontinuous, involving short successive steps from field to field and region to region. To a limited extent it is also continuous, involving longer, unbroken jumps from island to island over intervening seas" (147). This conclusion probably is applicable, at least in part, to many downy mildews whose conidia are quickly killed by high temperature, low humidity or sunlight.

Nevertheless, there could be considerable extension of even such diseases as downy mildews from south to north or north to south, depending on the hemisphere, in a single season if susceptible crops were grown during successive periods without too great interruption in space and time. Even though the infection may spread in short waves, the area covered in a single season may be extensive if all factors affecting the spread and development of the disease are conjointly favorable.

d. Endothia parasitica. The two pathogens just discussed have an advantage over many others because their spores are either produced or liberated, or both, when there is abundant moisture to facilitate infection. This is true, of course, of many other fungi also. As an example, the chestnut blight fungus, *Endothia parasitica* (Murr.) Clint., liberates ascospores during or after rain. Although spores were not caught more than 389 feet from known sources, the maximum number were caught when expulsion from asci was known to be going on and when moisture conditions were therefore favorable for infection (44).

e. Hypoxylon pruinatum. Recently it has been shown that ascospores of *Hypoxylon pruinatum* (Kl.) Cke., which causes a canker on poplars, are most abundant in the air during rainy periods, although free water is not so necessary for detachment of conidia as it is for discharge of ascospores. No ascospores were discharged when perithecial stromata were kept for 21 days in an atmosphere with the relative humidity near saturation. After soaking stromata from the same source in water for half an hour, however, ascospore discharge began within an hour, and spores were deposited at an average rate of 407 per square centimeter of surface for three hours in a nearly saturated atmosphere. It was shown in this investigation not only that spores were commonly present in the air from spring to fall in certain poplar plots in Wisconsin in which there were infected poplars, but that 80% of the new cankers that appeared in 1942 and 1943 were "down wind from and facing toward the old fruiting infections" (42).

Not all pathogenic fungi discharge their spores or have them ready for liberation when weather favors infection, as in the examples given above. With such pathogens local dissemination presents no serious problems with respect to the longevity of spores, and there is less risk of wastage because of inaccessibility of proper host plants than when the spores are liberated or carried away mechanically by dry air. The chance of long-distance dissemination is, in any case, reduced if spores are formed or liberated only during periods of wet weather. Possibly, therefore, what appears to be advantageous with respect to spore formation, liberation and opportunity for quick germination and infection, is disadvantageous with respect to short-time, long-distance dissemination, as distinguished from extension of a disease over a wide area as a result of a series of discontinuous local extensions. And too much generalization is dangerous in any case because the different kinds of spores produced by pleomorphic fungi do not necessarily behave alike.

f. Gymnosporangium juniperi-virginianae. In considering further the reasons why some fungi do not spread long distances in a short time, it is apparent that there may be several restrictive factors. *Gymnosporangium juniperi-virginianae* Schw., which causes apple rust, is a good example. Despite the production of enormous numbers of basidiospores and aeciospores, effective dissemination is only local. The fungus is heteroecious and overwinters in the well known cedar galls on red cedar, *Juniperus virginiana.* During spring or early summer rains these galls send out gelatinous "spore horns" bearing the teliospores which germinate *in situ* and produce basidiospores or sporidia that are forcibly abjected from the promycelia and are carried by the wind to apples where they cause infection resulting in the development of the aecial stage. The aeciospores are shot out from the aecial cups and are distributed by the wind, but they can infect only the red cedar, not the apple. There is, therefore, no repeating stage, and the disease can exist only if red cedars and apples are grown near each other. Although a single cedar gall is reputed to produce about 7.5 billion sporidia (65), the sporidia are relatively short-lived and cause abundant infection on apples only within a mile, depending on the position of the red cedars and apples with respect to elevation, screening vegetation and other factors affecting the chances of large numbers of spores reaching the apples (66). Although apples are occasionally infected ten or 15 miles from red cedars, the infection usually is very light more than a mile from an abundant source of inoculum; and severe infection usually is restricted to a radius of about one mile from them (39, 47, 97). It seems evident that the rate of diffusion of spores is very rapid, even though they are produced a considerable distance above the ground where the wind has easy access to them. Despite the profuse production of spores, the apple rust fungus is not well adapted to more than local dissemination. The disadvantages are the limited distances over which the sporidia are effectively disseminated, the relatively short time during which any individual cedar gall remains active, the lack of a repeating stage on either host, and the long period of incubation on the red cedar, lasting from spring or early summer to the second spring following. Another handicap to success is the relatively small and scattered population of red cedars in many apple-growing regions. Although the aeciospores produced on apples probably are carried considerable distances, the chances are slight that they will reach red cedars in sufficient quantity to cause abundant infection at a long distance from the apples. From the orchardists' standpoint it is fortunate that *Gymnosporangium* has these defects, for control of the disease is relatively simple in most regions.

g. Cronartium ribicola Fischer, the cause of white-pine blister-rust, also is handicapped despite the prodigious numbers of aeciospores that often are produced on pines. Were the aecial stage a repeating stage so that infection could spread directly from pine to pine, the situation with respect to control would be hopeless. But the aeciospores do not infect pines and are therefore harmless unless carried to currants and gooseberries, *Ribes* spp., on which they can cause infection that results in the production of urediospores. These can again infect certain kinds of currants and gooseberries and thus spread the disease until teliospore formation ends the activity of the pathogen on *Ribes*. The teliospores germinate by sending out a promycelium that produces sporidia whose effective dissemination seldom exceeds one mile, probably because they are short-lived (110), because they are produced on low-growing shrubs, and because of the screening effect of vegetation between or above them and pines. Because of this limited dissemination, white pines usually can be protected by destroying susceptible *Ribes* bushes within 900 feet, the exact distance depending on a number of variables, including the species of *Ribes* involved. But this is not the whole story. It is known that the aeciospores, often produced on lofty pine trees, can be carried long distances by wind, and the rust can therefore extend its geographic range by spreading from pines to *Ribes*. In the Pacific Northwest the disease has been frequently found on *Ribes* 100 to 200 miles from infected pines, and there is convincing circumstantial evidence that in favorable seasons the aeciospores may cause infection 300 to 400 miles from their place of origin (82).

Thus *Cronartium ribicola* may extend its geographic range considerable distances in one season through the medium of wind-borne aeciospores. Its establishment in the new region will, of course, depend on the presence and proximity to each other of the right kinds of *Ribes* and pines and on suitable weather conditions (88). Infection of *Ribes* is only the first step; the second may or may not be possible. In any case the wind has done its part.

Clearly, then, *Cronartium ribicola* has a dual nature as concerns its aerobiologic relations; from a practical standpoint this is important. The effective dissemination of sporidia from *Ribes* to pines is local and there is no repeating stage on pines; hence local eradication of *Ribes* is effective in protecting pine stands. Because of long-distance dissemination of aeciospores, however, the geographic range can quickly be extended from a few miles to several hundred miles into areas where there is appropriate association of host plants and favorable weather conditions for rust development. Were currants and gooseberries the principal economic hosts, their protection would be far more difficult. In fact, this situation has existed to a limited extent in certain European countries, especially England, where the cultivated black currant was considered more valuable than the small number of introduced white pines, *Pinus strobus* (109). As concerns the extension of its geographic range in North America, *Cronartium ribicola* probably was aided more by man than by wind, despite the conclusive evidence regarding long-distance wind dissemination of aeciospores. This certainly is true of its original importation into the United States on nursery stock and its distribution across barriers such as the Great Plains, where for hundreds of miles the relative absence of susceptible pines and *Ribes* would have interposed a barrier to the spread of the disease from the eastern white pine across to those areas of the west where there again is a concentration of susceptible pines and *Ribes*. The present verdict must be that man carried the pathogen from region to region on infected nursery stock but that the wind has effectively aided the pathogen in spreading from area to area within these regions.

None of the pathogens so far considered is known to be disseminated effectively over extensive areas in one season, at least in sufficient quantity to cause widespread and devastating regional epidemics. Most of them have certain handicaps which prevent them from accomplishing this. This is true also of many smut fungi which are well adapted to wind dissemination because of the large numbers of spores produced and their relative resistance to unfavorable conditions. That large numbers of smut spores are carried considerable distances by wind is clear from observations and the results of spore-trapping experiments (45, 54, 123). But many smut fungi are handicapped by being able to cause infection only in certain host plant organs or at a certain stage of development; therefore the time during which they can cause infection is limited.

h. Ustilago tritici and *U. nuda* (Jens.) K and S. Spores of loose smuts of wheat and barley are well adapted to wind dissemination. They are produced in large numbers, converting affected heads into powdery masses of spores that are easily carried by the wind. The spores are small, about seven microns in diameter, and retain their viability for months, even under unfavorable conditions (111), but they can cause infection only in the young ovaries at flowering time, a period of only a few days (90). The hyphae then remain in the seed, and spores are not again formed until almost a year later. Observations and experiments indicate that the range of effective dissemination is not great, usually not more than a few hundred yards (34). This probably is not because the spores are not widely disseminated; rather it is because of the limitations of time and space within which infection can occur, because of the long time that elapses between infection and sporulation, and probably also because of the fact that these smuts do not produce any sporidia, which are produced in enormous numbers by some smut fungi.

There is, indeed, evidence that spores of these smuts are widely disseminated by the wind, but it is again a question of effective dissemination. All evidence indicates that the rate of dispersal of spores is rather rapid under ordinary conditions. With fungi that can attack any above-ground parts of plants and that multiply rapidly because of a short incubation period and abundant sporulation, this is not a great handicap, as even a few spores may be the means of starting infection centers far from their place of origin. But it is very important for smuts like those in question because the chances are rather slight that spores will be deposited on ovaries of particular varieties of wheat or barley, respectively. And even if occasional ones do succeed in causing infection at considerable distances from their starting place, there is no secondary infection and consequent spread of the disease in the same growing season. In general, the smaller the infection court and the shorter the period of susceptibility, the more numerous must be the spores to ensure infection. Moreover, although wheat and barley are grown from southern Mexico to Canada, rapid northward extension would be difficult because the time of spore dispersal in one zone does not necessarily coincide with the period of host susceptibility in another.

Several practical problems are associated with the dissemination of loose smuts of wheat and barley. The modified hot water treatment which kills the smut within the seeds is cumbersome and is usually used on a treated seed-plot basis. How far, then, must the seed plot be from ordinary fields sown to non-treated seed in order to protect the seed plot from infection? In general, a few hundred yards to a quarter of a mile appears to be sufficient. Another important question arises in connection with the possibility of long-distance dissemination of spores of various physiologic races. Both species comprise races with different degrees of virulence for different varieties, an important fact in breeding for smut resistance. It is possible, but not demonstrated, that the races may extend their geographic range gradually, during several growing seasons, even though the distance covered in a single year might be short. Rapid, long-distance distribution, however, is more likely to result from distribution of infected seed.

i. Urocystis tritici. Many other smut fungi are similarly handicapped. Although they produce large numbers of spores that are disseminated by the wind, as has been mentioned for the bunt fungi, *Tilletia* spp., the spores are disseminated principally during harvest or threshing when plants are not susceptible to infection. Although there are local conditions which permit air-borne spores that fall on the ground to cause infection (46), such smut fungi as cause infection of young seedlings only are not spread principally by the wind, even though their spores may be carried considerable distances and in considerable numbers by this agency. That geographic extension of diseases may be limited, even though spores of the pathogen are wind borne, is shown further by the history of flag smut of wheat, *Urocystis tritici,* in the United States. The disease has been present for about 25 years on at least one very susceptible variety in certain sections of Illinois, Missouri and Kansas, but has not been found in the spring-wheat area farther north. Although large numbers of spores are produced and undoubtedly are carried by the wind, they are produced when wheat is not in susceptible condition. In any case, most of the hard red spring-wheats grown during the past 20 years are resistant; hence there are two barriers to effective wind dissemination.

j. Ustilago zeae and *Sorosporium reilianum.* Some smut fungi that attack crop plants seem well adapted to effective wind dissemination, notably corn smut, *Ustilago zeae,* and head smut of sorghum, *Sorosporium reilianum* (Kuehn) McAlp. It already has been mentioned that *U. zeae* produces countless billions of spores, and it is known that they are commonly present in the air. Young tissues of corn plants are susceptible during the entire vegetative period of the corn plant, and infection results principally from air-borne spores, as is evident from observations, experiments and the fact that seed treatment is of little value in controlling the disease. Moreover, the incubation period is relatively short, only two or three weeks, so that many successive crops of spores can be produced during the growing season. The spores are relatively small, about ten microns in diameter, and do not lose their viability easily. The senior writer has found spores still viable after seven years storage in the laboratory, and they usually germinate well even after having been exposed to a Minnesota winter. Yet there is but little evidence regarding long-distance dissemination. It is true that there have been reports of smut in corn plots many miles from corn fields, but there always is the chance that spores may have been carried with seed. Although not entirely conclusive, there is some evidence that there may be considerable localization of races of *U. zeae.* This fungus comprises an indefinitely large number of haploid biotypes that differ from each other in many characters, including sex, pathogenicity and various physiologic and cultural characters (121). In limited experiments by one of the writers it appeared that there was a tendency for lines from various regions to fall into groups according to the locality from which obtained. Supporting evidence is the fact that collections of chlamydospores from a number of states were consistently different in pathogenic effects on several varieties of corn inoculated three successive years in the field. This does not preclude the possibility of long distance dissemination, but does indicate that it is not so common as to prevent at least a certain degree of localization of types. Unfortunately, however, there are so many haploid lines within the species and new ones are produced in such profusion through mutation and recombination, that results of studies such as these are indicative rather than conclusive. All that can be said with certainty, therefore, is that both the chlamydospores and sporidia are commonly carried by the wind, but the distance of effective dissemination is still conjectural.

LONG-DISTANCE DISSEMINATION

Although several of the factors discussed above effectively limit the rapid inter-regional spread of certain diseases in a single season, they by no means preclude the possibility of occasional, or rare, but effective, dissemination of one or a few spores of a virulent race or species of a pathogen from one region or continent to another. Given years or decades, such a pathogen might build up to where it would cause destructive epidemics. This admittedly is a long chance, but some of the plant pathogenic fungi seem admirably suited to take advantage of such long chances, and they are by no means pressed for time. Our present facilities and techniques for the study of air-borne plant pathogens scarcely permit the critical investigation of what may seem like outside probabilities; eventually, however, we must devise ways and means of determining whether such dissemination occurs. Should a destructive plant pathogen be effectively disseminated in this way only once in a century, practical considerations alone would justify the work necessary to detect it. And plant disease fungi are not the only important micro-organisms which may spread in this fashion, although they are the only ones under consideration here (32).

None of the pathogens so far considered is known to cause epidemics over wide areas and in a short time as a result of wind-borne inoculum. It is evident that mere dissemination of spores is only a first step in effective dissemination, in the establishment and multiplication of inoculum.

All things considered, some of the downy mildews, powdery mildews and cereal rusts are best suited to rapid dissemination and multiplication and consequent development of regional epidemics in a short time. There is some circumstantial evidence that certain downy mildews may spread considerable distances northward from southern United States in a single season (31, 140, 141), but the evidence is most conclusive in some of the cereal rusts, especially *Puccinia graminis* Pers.

Stem-Rust of Wheat

Puccinia graminis tritici (Pers.) Eriks. and Henn., the cause of stem rust of wheat, barley and many other grasses, is one of the best examples of a pathogen that can become devastatingly epidemic over a wide area and in a short time, even though there may be relatively little inoculum at the beginning of the growing season. One of the most favorable areas for study of it comprises Mexico, the Mississippi Basin of the United States, and the prairie provinces of Canada. Wheat is grown from south central Mexico to the prairie provinces of Canada, almost without interruption, except for certain areas in Mexico and southern Texas. For a distance of about 2,500 miles there is an almost continuous population of rust-susceptible plants, including the wheat itself, barley and other susceptible grasses. Because of differences in latitude and elevation there are susceptible plants somewhere in this great area at all seasons of the year, and there are no topographic barriers between them. The wind has a free field and a vast one. It blows from south to north and from north to south, often carrying countless numbers of spores and other minute objects hundreds of miles within a few hours.

Necessity and opportunity have motivated studies of the seasonal development of stem rust of wheat in the great wheat-growing regions of North America. The disease has frequently caused such destruction epidemics, especially in the hard red spring-wheat area of the upper Mississippi Basin of the United States and in the prairie provinces of Canada, that it became imperative to ascertain the source of the rust as a basis for control measures. Some studies were begun soon after the destructive epidemic of 1904, and the senior author began observations and experiments in Minnesota in 1909. The scope of the work was expanded and considerable progress was made prior to the epidemic of 1916. Following this economic disaster, epidemiology studies were begun on a regional basis, from Texas northward, and they have been continued since that time by the United States Department of Agriculture in cooperation with the Minnesota Agricultural Experiment Station and various other experiment stations. Cooperative studies with the Mexican Department of Agriculture and similar studies by Canadian investigators, have elucidated the general features of rust development and have convicted the wind of responsibility for rapid and widespread dissemination of inoculum, as had been suggested by Freeman and Johnson, and others (35, 113).

Three generalizations, all based on extensive observations and experiments, are basic to an understanding of the rôle of the wind in disseminating spores of wheat stem-rust in the region under consideration:

a. The uredial stage, the only repeating stage, does not survive the winter north of Texas, except occasionally and locally (2, 115).

b. The aecial stage develops on barberries regularly and abundantly only as far south as the 39th parallel, except in mountain regions in eastern and western United States, where elevation is a substitute for latitude (100, 125).

c. The uredial stage does not normally persist through the summer in Texas and northeastern Mexico, except occasionally in mountain valleys in northern Mexico (137).

Urediospores may retain their viability for several months at moderate temperature and humidity. They are injured by direct sunlight, but only after exposure for about 15 hours or longer (51). Aeciospores can retain their viability three to four weeks under favorable conditions (23). Urediospores may germinate in less than an hour, and aeciospores in about an hour at a favorable temperature.

The incubation period of the uredial stage ranges from six days to three weeks, depending on temperature and light, under conditions that are likely to prevail during the growing season (78, 127). There often are four or five uredial generations during the growth period of wheat following initial infection, thus enabling the rust to multiply rapidly and spread widely from small beginnings. A single uredium contains 50 thousand to 400 thousand spores, and there are countless billions in a heavily rusted infection center a few rods in diameter.

There are at least six varieties of *Puccinia graminis*, differing in pathogenicity on small grains and other grasses. The urediospores and aeciospores of *P. graminis* can be recognized easily as belonging to the species, but spores of the varieties that attack cereal grains cannot be distinguished with certainty on spore traps. *P. graminis tritici* comprises about 200 known parasitic races, differing in pathogenicity on different varieties of wheat (130). These races cannot be distinguished readily by spore characters.

There naturally are many variations in the pattern of rust development within so extensive an area as that under consideration. In general, however, the north and south often are complementary. As the uredial stage does not survive the winters in the north, initial infection must result either from aeciospores produced on *Berberis* spp. within the region or from urediospores blown in from the south. And since the aecial stage does not develop in the south and the uredial stage does not persist there through the summer, there must be an outside source of inoculum to infect winter wheat in the southern region in the fall. These needs are well supplied by the wind in many seasons: "Northers" carry urediospores from north to south in the fall, and south winds carry them from south to north in the spring and early summer. The distance of effective dissemination at a given time depends on the abundance of spores, the presence of susceptible hosts, and weather conditions affecting infection after the spores have been deposited on the plants. Sometimes rust spreads in relatively short, successive waves, and sometimes a single wave of infection may cover several hundred miles. Sometimes the number of wind-borne spores is so large that abundant and uniform infection may break out over a large area at one time, and sometimes the number is so small that only occasional plants in a field become infected. Regardless of the pattern of development, it now is known definitely that ruinous epidemics can spread in less than two months from northern Mexico and Texas northward through the United States and far into Canada, a distance of 2,000 miles. Here the wind is at its destructive worst in disseminating a plant pathogen over an area within which about a billion bushels of wheat are produced annually (49, 62).

The course of development of epidemics, which sometimes almost literally have been borne on the wings of the wind, has been traced with considerable accuracy for more than 20 years. Three principal methods are used: spore trapping, observations on the development of the rust in the field from south to north, and determination of the geographic distribution of physiologic races. Although results obtained by any one of the three methods alone might furnish reliable data in some cases, conclusions sometimes might be erroneous unless all three were used. The results of spore trapping may be reliable when there are many spores in the air, but when too few spores are present to permit accurate sampling, negative results cannot be conclusive. Thus, early and light infection sometimes has occurred in some regions before spores were caught. If reliance is placed merely on the sequence of rust development in different north-to-south and east-to-west zones, confusion sometimes results because there may be several sources of inoculum. The distribution of races may or may not be such as to furnish evidence regarding the origin of inoculum. Combination and adequate use of all three methods, however, supplemented by study of air movement, rainfall, dews and temperature, usually make it possible to chart the progress of rust development with considerable certainty.

Although stem rust epidemiology studies were begun on an extensive scale in 1917, it was not until 1923 that entirely satisfactory evidence was obtained of south-to-north movement over a wide front.[6] Fragmentary evidence was available and circumstantial evidence was strong, but basic information regarding necessary facts and procedures was lacking. Moreover, the development of local and regional epidemics from the millions of barberry bushes in the northern states sometimes made it impossible to trace the original source of the inoculum in this area. By 1923, however, enough barberries had been eradicated in an eradication campaign begun in 1918, to simplify the situation somewhat with respect to recognition of sources of rust.

Histories of wide-spread stem-rust epidemics. In 1923 the uredial stage of wheat stem-rust overwintered in southern Texas and northern Mexico, but not in northern Texas and northward, as determined by extensive observations and experiments throughout the fall and winter in 15 states, from Texas to Minnesota and from Colorado and Montana to Tennessee and Ohio. By April 20, 1923, rust was fairly abundant in some fields in southern Texas and northern Mexico and spores were caught on vaselined glass slides exposed near the ground at Laredo, Texas, at about the same time. On May 4, spores were caught on slides exposed from an airplane flying at an altitude of 1,500 feet between Laredo and San Antonio, Texas. There was, therefore, northward dissemination of spores during late April and early May, and probably earlier. By May 10 rust infection had extended into southern Oklahoma, by June 4 it had reached Kansas, southern Nebraska and southern Illinois, following spore dissemination about May 20. Spores were caught at St. Joseph, Missouri, on May 31, and by June 22 rust was found on wheat as far north as southern South Dakota and southern Minnesota; and by July 1 it had reached the Canadian border. This extension of rust from southern Texas to Canada required about two months and proceeded northward in five successive waves. Although there was light to heavy infection on barberries at various places from Oklahoma northward, the local epidemics on grains and other grasses from this source could usually be distinguished from the general and relatively uniform infection resulting from spores carried northward by south winds. Moreover, the results of spore trapping by means of vaselined microscope slides exposed systematically near the ground at 38 stations, distributed from southern Texas to northern North Dakota and Minnesota and from Colorado and Montana to Ohio, confirmed the conclusions regarding south-to-north dissemination of spores, even though the time and method of exposure of traps were not always optimum. Finally, the distribution of physiologic races 11, 17 and 21 supported the evidence obtained by the other methods.

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[6] Statements regarding the epidemiology of cereal rusts are made on the basis of data obtained by the senior author and associates in a long-time rust epidemiology project carried on cooperatively by the United States Department of Agriculture and the Minnesota Agricultural Experiment Station.

There was, therefore, conclusive evidence that urediospores of *Puccinia graminis tritici* were disseminated from northern Mexico and southern Texas to the Canadian boundary during the growing season of 1923. Even though the relative importance of this source of rust, and that resulting from the aecial stage, could not be determined precisely in the more northern states, the results show definitely that there would have been widespread development of rust in the area bounded by the Rocky Mountains on the west and the Mississippi River on the east, with generally lighter infection east of the river, had there been no other source of initial inoculum than that from the far south.

Again in 1924 there was evidence of rust movement from southern Texas northward, although it was a light rust year. The uredial stage again survived the winter on wheat in at least a few fields as far north as Waco, Texas. As in 1923, vaselined slides were exposed at many stations in the United States. There were spores in the air in southern Texas on April 20, and by May 27 they were caught in northern Oklahoma. During the first week of June they were caught at various stations in Kansas, Missouri, Kentucky and Illinois; during the second week in June some were caught in Nebraska and western South Dakota; and during late June and early July they were carried to the Canadian border and no doubt far beyond. Rust development on wheat was correlated well with spore distribution, even though the earliest spore movement was not always detected. As in the previous year, races 11, 17 and 21 were found in Texas and northward. Development of rust was generally late and slow, and it required almost three months to spread from central Texas to Canada, in seven fairly well defined waves. It was therefore considerably slower than in 1923 because of generally subnormal temperature, but the spread was nevertheless clear, again especially west of the Mississippi River. However, rust sometimes moves far faster than in 1923 and 1924; this was true in 1925 (126).

The evidences of overwintering of the uredial stage of wheat stemrust in Texas in 1925 were not so conclusive as in some previous years. In fact, it is probable that much of the rust in Texas came from northern Mexico. Nevertheless, urediospores were caught at Dallas, Texas, about the middle of May; at Little Rock, Arkansas, on May 12; at Cairo, Illinois, on May 20; and at Springfield, Missouri, at about the same time. A short time later, during the first few days of June, spores were found on slides exposed at Hannibal, Missouri; Concordia, Kansas; Lincoln, Nebraska; and North Platte, Nebraska.

Rust developed on wheat as far north as southern Kansas by May 25; and by June 1 it was general but not heavy in Texas, Oklahoma, southern Kansas, southeastern Missouri and southern Illinois. It could not be found, however, in southern Nebraska, nor northward, despite persistent search. There was nothing alarming in the situation, as infection seemed light. But the wind did so thorough a job of disseminating what spores there were that the rust greatly extended its range suddenly and spectacularly.

Conditions were favorable for pronounced convectional currents over Texas from June 4 to June 9 and over Oklahoma from June 2 to June 7. On June 1 there was a strong surface wind from Texas, almost due northward to North Dakota; and from June 2 to June 7 there was a general strong air drift from Texas to Nebraska. During most of this period the movement was northward over the Minnesota-North Dakota region also. For the first four days of this period, wind velocity averaged 19.5 miles an hour at Oklahoma City, Oklahoma; 24 miles at Wichita, Kansas; 26 miles at Sioux City, Iowa; and 17 miles at Devils Lake, North Dakota. There was precipitation over most of Nebraska, the Dakotas and northwestern Minnesota each day of the first week of June. Rusted wheat was then found in southern Nebraska on June 8, and on June 10 it was general in the areas surveyed in northern Nebraska. Farther east at some places in Minnesota the strong south winds were not followed by rain, and, despite the spore shower, rust did not become established. A thorough study of rust development from north-central Kansas northward through Nebraska, the Dakotas, northwestern Minnesota and northeastern Montana showed clearly that infection had taken place in virtually all fields of susceptible wheats in this entire area almost simultaneously, only two or three days later near the northern limit than in the southern. This would be expected on the basis of relative time required for spores to traverse the 600 miles from south to north. Differences in time of precipitation would also cause some difference in time of initial infection, and differences in temperature would make a difference in length of the incubation period.

The number of spores that caused infection decreased northward, as is shown by the fact that about 7% of the culms of susceptible varieties were infected in Nebraska, about 4% in southern South Dakota and 2% in the northern part of the state, about 1% in most of North Dakota, decreasing to zero a few miles from the Canadian border, and decreasing also westward in Montana. From this early, light infection, infection centers developed more or less rapidly, depending on local rainfall and temperature. In some areas where there was hot, humid weather, at least four subsequent uredial generations developed, and the result was heavy rust and severe damage to wheat in an extensive area embracing parts of the Dakotas and Minnesota.

In 1925, then, urediospores of *Puccinia graminis tritici* were effectively disseminated within the first week in June over an area about 600 miles long and more than 400 miles wide, an area of at least a quarter of a million square miles. The earliness of this great "spore shower" made it possible to fix its source easily and with unquestionable certainty. It came even before the uredial stage had developed appreciably on grains and grasses near barberries; it came at a time when there definitely was no other possible source of abundant inoculum north of central Kansas. The wind movements were ideal for dissemination of spores over a vast, rust-free area; the general precipitation over much of the area favored infection; and the subsequent development of rust, with the prevalence decreasing rather uniformly northward and westward, give one of the simplest and clearest pictures on record of widespread dissemination of stem rust in a short time. The area probably was larger than that described, as only that portion of the picture completely free from any possible blur has been shown. In 1925 the rust wrote its own aerobiological story with unusual lucidity and legibility. There have been more catastrophic rust movements and developments in this area, but few that were as clearly legible. Indeed, the wheat-growing area northward from Kansas in 1925 was like a large agar plate on which the number of fungus colonies decreased proportionately with the distance from a given source of spores. And the medium, wheat, was a highly selective one, permitting development of *P. graminis tritici* only. Mere numbers of urediospores on slides could never have told the story as it was told in 1925. And had confirmatory evidence been needed, it would have been supplied by the distribution of races 11 and 36 from Texas northward.

From the aerobiologic standpoint the epidemic of stem rust on wheat inTexas and the wheat growing regions of the Upper Mississippi Basin and western Canada in 1935 is particularly significant, because the ground work, or air work, for the epidemic was laid in the fall of 1934, when spores were carried southward on October 10 and again from November 10 to 12. Rains followed these north-to-south air movements, and 64% of the winter wheat fields in Oklahoma became infected (119). Evidence of this spore shower was found also on oats from southeastern Kansas to central Texas. Although the rust was threatened with extinction in Texas during the winter, some survived. A late spring and superabundant rainfall in northern Texas in May favored extraordinarily heavy development of rust; spores were disseminated early and encountered favorable conditions because of late wheat in parts of Kansas and northward; weather conditions were favorable for rust development in the spring-wheat region; and one of the worst epidemics on record destroyed about 135 million bushels of wheat in Minnesota, South Dakota and North Dakota, despite the fact that a hitherto resistant variety, Ceres, had largely replaced the susceptible Marquis in that region.

In 1935 rust was well developed in Texas by May 10, and there were pronounced air movements from southern Texas northward into the spring-wheat area on May 11 and 12, followed by wind from western Texas northward and eastward into Missouri on May 13. Spores were found on May 14 on vaselined slides exposed at four places in Nebraska, and subsequent observations on rust development showed that there had been a uniform spore shower over Oklahoma and eastern Kansas, and as far north as St. Joseph, Missouri, at that time. On May 17 and several days thereafter spores were caught on slides in eastern Nebraska when winds were from the south. The highest number was 720 per square foot during a 24-hour period. On May 24, 25 and 26 wind blew from Texas into South Dakota, and spores were again trapped in eastern Nebraska and on each of the three days at Brookings, South Dakota. Spores were deposited on slides at Fargo, North Dakota, early in June, during periods of south winds; and on June 15 they were deposited at the rate of 4,800 per square foot in eastern Nebraska and 288 per square foot at Fargo. Again from the 22d to 24th and subsequently, large numbers were caught, 15,000 at Fargo, more than 10,000 at Brookings, and about 2,500 at St. Paul. A fairly heavy spore shower fell in southern Manitoba on June 23 and 24 (26). By July 5 the rust was epidemic and well on its way to destroying the wheat crop. The numbers of spores trapped after this time became merely a matter of general interest, but, as an indication of their abundance, they were deposited at the rate of 921,600 per square foot during 24 hours at Waverly, Nebraska, on June 30, and 981,300 at Fargo, North Dakota, on July 25.

Nothing could be clearer than the sequence of events in 1935: the north-to-south movement of rust in two principal waves in the fall of 1934; its subsequent establishment over a wide area from Oklahoma through Texas and into northern Mexico; the struggle of the uredial stage through the winter; the development of an extraordinarily heavy epidemic in Texas in the spring; the south-to-north spore-bearing winds; the subsequent establishment and rapid multiplication of the rust in Kansas, Nebraska and northward; and the virtual infestation of air and wheat by urediospores that were so numerous as to be deposited on glass slides at the rate of nearly a million per square foot in 24 hours. The distribution of races 11, 34 and 56, the last a relative newcomer among races, and observations by Canadian pathologists, completed an air-tight case against the wind, and convicted it of spreading destruction that was disastrous in its effects (26, 56, 116).

The main features of south-to-north movement of wheat stem-rust in the United States have been given for four years as case histories, as they illustrate the variations in sequence of events, even though the essential principles are the same. There are still other variations in development, as in 1937 when favorable winds carried large numbers of spores not only northward but eastward through Missouri, Illinois, Indiana, and as far as eastern Ohio. The south-to-north movement is usual, although for many reasons it results in destructive and widespread epidemics only when all factors for rust development operate in conjunction; but the effective eastward spore movement, such as that of 1937, is unusual. The eastern and western limits of the "spore movement front" vary with the season, but this is more pertinent to a consideration of rust epidemiology in general than to long-distance dissemination of spores.

Practically, with epidemics starting as they often do from small beginnings, it is of utmost importance to eliminate as much inoculum as possible through eradication of alternate hosts in the north and the development of resistant varieties of grains, not only for the north where rust does its greatest damage, but also for the south where the rust overwinters and multiplies.

In addition to south-to-north movement of wheat stem-rust from northern Mexico into Canada, there may be many local or regional epidemics resulting from the aecial stage on barberries. There is abundant evidence that numerous local epidemics extending for a few rods to several miles from the bushes may often develop relatively early in the season (64, 124). These epidemics may spread in successive waves, as is true of the south-to-north movement. The billions of aeciospores produced on barberry bushes in northern United States may cause infection directly on grains and other grasses during April, May or June, depending upon the region and the season. The urediospores resulting from the first infections may then be carried long distances, just as are those from the fields in the south. In some localities and years, therefore, it is difficult to distinguish between the two sources of inoculum. Epidemics have been known to spread a hundred miles or more from barberries (103). Even though they may not spread as far as this directly, epidemics from bushes in different locations often have been known to coalesce in the northern half of the United States (124).

Naturally, there is no tag on urediospores caught on slides to tell whether they came from the south or from barberries in the north. Spore-trapping results, therefore, must be interpreted on the basis of known facts with respect to potential sources of inoculum, with respect to wind direction and velocity, and with respect to the particular varieties or races of rust present. This is especially true in connection with the development of epidemics near barberries, because the primary infection often is on grasses that may be susceptible to more than one variety of *P. graminis* Pers.

The extent of infection resulting from barberries can not always be measured by the degree of infection near the bushes. The aeciospores themselves are sometimes carried long distances after reaching the upper air currents, as they have been caught at altitudes of 7,000 feet and are known to retain their viability for three to four weeks at moderate temperatures and moderate humidity. It is entirely possible, therefore, that small centers of infection may result from aeciospores or from urediospores formed near barberries at considerable distances from the place where the inoculum was produced. Some idea of the numbers of spores carried by the wind from barberry bushes or from infected grains and grasses near them can be obtained by considering the number of aeciospores caught on slides that were exposed four feet above the ground near a heavily infected barberry bush in southern Minnesota in late May. On one slide exposed two days three feet from a bush there were aeciospores at the rate of 7,000,000 per square foot; on a slide exposed three days at 90 feet from the bush there were 45,000 per square foot; a slide exposed for a week at a distance of ⅝ mile trapped 144,000 aeciospores per square foot; and on a slide exposed for a week one mile away from the bush, aeciospores were counted at the rate of 100,000 per square foot (114). Theoretically, then, enough inoculum could be furnished by barberries to cause general and widespread infection in the northern states. In reality this was true before barberry eradication had been carried to its present status. But even at present, widespread epidemics can result from areas where large numbers of barberries are known to exist.

Regional epidemics extending from western Virginia, and probably from contiguous areas in West Virginia, spread northward and westward in 1942 and 1943 (131). In both years bushes of *Berberis canadensis* Mill., which are very abundant in the Virginias, became heavily rusted about May 15. The rust then spread to wheat and to other grasses, and produced very heavy local epidemics that finally coalesced into a general epidemic in the area where the barberries were most numerous. During the first half of June, southeast winds followed by rains carried this rust through much of Ohio and Indiana, and in 1942 as far as southern Michigan and northeastern Illinois. There was some southward and southwestward spread also, but the principal epidemic developed toward the west-northwest, along the path of the winds. The course of development of these regional epidemics was made clear by spore trapping experiments, study of air movements, observations on the early infections on wheat, and finally by a study of the relative prevalence of physiologic races in this area as compared with that west of the Mississippi River, where there was a south-to-north movement. It was found that race 38 was so predominant in the eastern area, and was so scarce west of the Mississippi River, that the results of the survey confirm completely the results of spore trapping and field observations on the development of rust on grains and grasses (132, 134).

Intra- and Inter-regional Dissemination. In all the epidemics discussed so far, the sequence of events has been rather clear. It can not be emphasized too often, however, that the situation is not so simple as cursory study might indicate. The United States could be divided into zones and sub-zones with respect to aerobiological relationships of the cereal rusts. The two major zones repeatedly referred to in describing epidemics are the southern and the northern. The southern zone includes northern Mexico, Texas, Oklahoma, at least part of Kansas, and areas at about the same latitude eastward and westward in the inter-mountain region, except where the zone line dips farther south in the Appalachian Mountains and the Rocky Mountains because the climatic conditions at high elevations are more like those of the north than those of the south. The southern zone, in general, is that in which the uredial stage of *Puccinia graminis* does not survive the summer, and the northern zone is that in which barberries become infected. These two major north and south zones could be subdivided by north-south lines. The area bounded by the Mississippi on the west and roughly by the Ohio on the south could be considered a sub-zone, as could the high plains from Texas northward. The Pacific Coast area could be considered as another zone, again divided into several sub-zones and into still smaller sub-zones in certain valleys. Naturally, therefore, generalizations are safe only on the basis of experience. It is not yet known how many years are required as an adequate sample. There is definite evidence that there may be intrazonal dissemination of spores and that the interzonal relationships are important only when a considerable number of factors operate in conjunction at the proper times and places. The wheat rust epidemic extending from the *Berberis canadensis* areas of the Virginias can be considered an intrazonal development. The sequence of events beginning in the fall of 1934 that led to the rust epidemic of 1935 illustrate interzonal development at its peak of biological perfection. The relative independence of certain zones or sub-zones from each other in certain years is perfectly clear. In 1943 there was far more stem rust on wheat than on oats in the region east of the Mississippi River, whereas west of the River stem rust of oats was much more prevalent than that on wheat, indicating separate origin of rust for the two regions. The epidemic of 1937, on the other hand, probably represents the most extensive interzonal development that has been studied in detail in the United States.

Rusts of Oats and Leaf Rusts of Wheat in the United States

The writers have attempted to illustrate certain principles developed in the study of wheat stem-rust, *Puccinia graminis tritici.* If further evidence were needed to confirm the conclusions reached, they could be furnished by adducing facts regarding similar epidemics of other kinds of rusts. As an example, a widespread epidemic of oat stem-rust, *P. graminis avenae,* developed in the Mississippi Basin in 1926. The sequence of events was just as clear as in the case of the wheat stem-rust epidemics already described. Similarly, an epidemic of crown rust of oats, *P. coronata avenae,* developed in 1927 and certain other years; and a widespread and destructive epidemic of leaf rust of wheat, *P. rubigo-vera tritici,* developed in 1938 (17, 18, 19, 30, 79, 122). There is almost a temptation to say that all cereal rusts may be widely disseminated by wind and become epidemic over extensive areas as a result of wind dissemination of spores. This has not been true, however, of stripe rust of wheat, *P. glumarum tritici* (Schm.) Eriks. and Henn. This rust sometimes develops very abundantly in Mexico, sometimes only about 100 miles or less from the Texas border. Susceptible varieties of wheat were planted a number of successive years at San Antonio, Texas, but did not become infected even in years when considerable infection of wheat stem-rust clearly resulted from spores blown into Texas from Mexico. There appears to be only one record of the occurrence of stripe rust in Texas[7]. Unfortunately the spores of *P. glumarum* are difficult to distinguish from certain other rust spores on slides. From results obtained in Germany (48), however, there can be no question that the urediospores of this rust are disseminated by wind, as are those of the other cereal rusts. The effective dissemination in certain parts of North America, however, is limited because of the sensitiveness of the urediospores to high temperature and desiccation (3, 70, 85). It has been pointed out that the geographic distribution of this rust is limited to areas or seasons of moderate temperatures. In addition to other possible factors (50, 104), it seems likely, therefore, that one of the main reasons why stripe rust does not develop in the principal wheat-growing areas in the Mississippi Basin is the exsistence of unfavorable meteorologic conditions rather than a lack of dissemination of inoculum.

Details regarding the development of regional stem rust epidemics have been given for Mexico and the United States only, and statements regarding Canada have been restricted to generalities. The conclusions, however, are based on detailed evidence obtained by Canadian investigators, who have concluded that, in general, rust in the prairie provinces develops as a result of inoculum originating in the United States and disseminated northward and westward by the wind (26, 91).

Cereal Rusts in Europe, North Africa and India

Dissemination in western Europe appears to be local (64, 112), but the interdependence of other regions with respect to development of cereal rusts has been pointed out, for example, Russia, India,

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[7] According to correspondence with E. S. McFadden, College Station, Texas, in spring of 1941. Reported by H. B. Humphrey in Plant Dis. Reporter **25**: 337. 1941.

Rumania and North Africa (16, 71, 72, 73, 101, 102, 105, 107). Details vary in the different areas. For example, the problem in India for many years was to explain the origin of rust inoculum after the rusts had been eliminated in the plains regions during the hot dry summers. Present evidence strongly indicates that the uredial stage survives in the northern hills and mountains and is then disseminated by wind from them to the principal wheat-growing areas of the plains. In some respects, therefore, the situation is somewhat similar to that in North America, where the difficulties involved in lack of oversummering of the uredial stage in the south and its failure to overwinter in the north are overcome to a considerable extent by interchange of inoculum between the two regions in the fall and in the spring. The situation in India is similar to that in Greece, except that in the latter a smaller area is involved. In some years, at least, the uredial stage of *Puccinia graminis* is eliminated from the plains of Greece because of high temperatures and absence of susceptible hosts. In the mountains, however, barberries, particularly *Berberis cretica* L., become rusted heavily during summer; the rust then spreads to grasses, where the uredial stage continues to develop and is then again blown back to the plains in the fall or early winter (112).

HOW FAR CAN VIABLE SPORES BE CARRIED BY WIND?

The question still remains as to the maximum distance of effective dissemination of inoculum of plant pathogens. Again the cereal rusts probably furnish the best evidence, because the source of inoculum is usually clearer than is true of many other fungi. As pointed out previously, viable spores of *Alternaria* and certain other genera of fungi are fairly common in the air at altitudes of 10,000 feet, and viable spores of many fungi have been caught at 5,000 feet or more. They have been taken to high altitudes in experimental balloons and have been found viable on their return to the earth (75). Moreover, spores have been collected in the arctic atmosphere at considerable distances from known sources (76). Always, however, there arises the question of effective dissemination. It seems perfectly safe to say that spores after once having attained altitudes up to 10,000 feet may be carried indefinite distances by mass air movements unless brought to earth by down currents or by rains or their equivalent. That spores of rusts can be carried in viable condition for several hundred miles under certain conditions and cause infection when deposited on susceptible plants seems clear from evidence already given. Viable urediospores have been caught at 5,000 feet near Norway House, Manitoba, when they must have been carried more or less horizontally at least 200 miles (2). There still is no definite evidence regarding effective dissemination across ocean barriers, although there is circumstantial evidence, because of the similarity of races of *Puccinia graminis tritici* in New Zealand and Australia, that inoculum may sometimes be interchanged between these two land areas, approximately 1,800 miles apart (145, 146). There is no evidence, however, that there is effective interchange of inoculum across the Pacific between Asia and North America, across the Atlantic between Europe and North America, or even between South America and North America. A very virulent race of *P. graminis tritici,* No. 189, has been known in the coastal region of Peru for a number of years but has never been found in North America (37). The air movements would not seem to be favorable for the dissemination of inoculum of this race northward to the North American continent, and yet it might happen, either by a long circuitous route or in shorter waves of infection and subsequent dissemination of inoculum at high altitudes in South America, with a subsequent rather long air journey between the wheat-growing regions of the highlands in northern South America and similar regions in Central America. Even mountain ranges sometimes seem to constitute a rather effective barrier against regular or frequent interchange of inoculum. As an example, in some years there is a regional development of stem rust of wheat in the Palouse district of western Washington resulting from development of the aecial stage on barberry bushes in the spring. A thorough study of the physiologic races in that area as compared with those in the wheat-growing areas east of the Continental Divide indicated that for a number of years at least there had been no important interchange of inoculum (134), because there is no correlation between the prevalence of physiologic races between the two regions. There seems to be a similar situation with respect to a lack of interchange of inoculum between the prairie provinces of Canada and British Columbia (84). Somewhat more difficult to explain is the lack of seasonal interchange of inoculum between wheat-growing regions of southern Mexico and those of northern

Mexico. Again the differences in the prevalence of physiologic races in the two areas have been so pronounced and consistent as to preclude the possibility of important irregular interchange of inoculum between the areas (137). In this case the direction of prevailing winds with respect to relative locations of wheat-growing areas may partly account for the lack of effective dissemination between the two areas. It is of course obvious that long-distance, effective dissemination between non-contiguous areas must depend upon winds blowing in the right direction at the right time. Moreover, the lack of fairly regular seasonal interchange does not mean that there is never any interchange. One of the most obvious precautions that must be taken in interpreting facts like the above is the relative susceptibility of varieties in different areas. It is known that races of *Puccinia graminis tritici, P. graminis avenae* and probably many other rusts may vary in seasonal prevalence (84, 133, 135, 146). Without appropriate consideration of this fact, the rôle of wind movements in dissemination of rust may easily be misinterpreted. For example, there are years in which the durum wheats of the Dakotas and northwestern Minnesota are in no danger whatsoever, regardless of the amount of inoculum blown from the south, merely because the inoculum does not include the race or races that can attack the durums. The durums, therefore, are susceptible in some years and resistant in others, depending upon the prevalence of different races of the pathogen. This is true of certain other varieties of wheat also, as well as of oats; and it probably is true of many other crop plants in their relations with many other pathogens.

DISSEMINATION OF PHYSIOLOGIC RACES OF PLANT PATHOGENS

Even though the wind may not be important in long-distance dissemination of inoculum of a pathogen in any given season, it may still be extremely important in extending the geographic range of pathogens or of physiologic races. Although this probably is true of many pathogens, it can best be illustrated with facts regarding *Puccinia graminis.* That new races are being produced continually as a result of recombinations in the sexual stage on barberry has been shown repeatedly (25, 86, 87, 128, 129, 144). Some of these races have but little survival value and never become established; others, however, that have no conspicuous weaknesses may become established, either quickly or slowly, depending upon wind dissemination of the spores and conditions favoring their establishment and survival. Race 56 of *P. graminis tritici* is one of the best examples of a race that extended its geographic range quickly (120, 135). In the United States it was first found in 1928, in Iowa, Nebraska and Kansas, in each case near barberries or in localities where barberries are known to rust. Although it probably was in existence before that year, it certainly was not present in more than trace amounts. The prevalence increased irregularly at first, then slowly but regularly until it became the predominant race in 1934. By that time it had extended its range to virtually all of the wheat-growing regions of North America except southern Mexico. Although there is no way of knowing exactly when the spores were blown from place to place, the wind is the only important agent of dissemination of rust and was clearly responsible for the extension of the geographic range of race 56. It undoubtedly was aided greatly by the mass air movements from north to south in the fall of 1934 and the subsequent air movements and favorable weather conditions that led to the epidemic of 1935. Despite the fact, however, that this race has been by far the most prevalent race in the United States since 1934, with the exception of one year, and has been established in northern Mexico since that year, it was not until 1938 that it was found in southern Mexico, and since that time it has been noted occasionally and sporadically under such conditions as to suggest that a few spores are occasionally blown into southern Mexico from northern Mexico. It has not survived in some of the localities in southern Mexico in which it was found in certain years, as it was not found in those localities in the succeeding year despite persistent search. That new or unusual races do not always become established easily or quickly is shown by the fact that certain virulent races have been found occasionally for a considerable period of years near barberries, and occasionally away from them, without having become generally established. Thus it has been pointed out that 16 races of *P. graminis tritici* were isolated in 1940 in the United States only from barberries or from rusted wheat in the area in which barberries commonly become rusted (136). None of these races was found in Mexico, Texas or Oklahoma, so that they were developed and distributed within the northern zone in the United States without having become established in the southern zone either in that year or subsequently. It seems likely that some of them may become established sooner or later, but a sequence of events somewhat like those of 1934–1935 probably will be necessary to permit extensive establishment. There has been a somewhat similar sequence of events in connection with certain races of *P. graminis avenae.* Races 8 and 10, for example, which are very virulent on some recently developed resistant varieties, were carried from the north to the south where the uredial stage survived the winter of 1942–43; and abundant inoculum was then blown northward in the summer of 1943 and again in 1944 (133). These general principles apply not only to new or unusual races but also to races that once were prevalent and then decreased in prevalence and shrank with respect to geographic range. This was conspicuously true of race 17 of *P. graminis tritici,* which was very common in the United States during certain years of the decade 1921–30, and then almost passed out of existence, increased slowly and somewhat irregularly from 1932 to 1939, and then increased rapidly until it comprised more than 50% of all the isolates in northern Mexico and the United States in 1941. The available evidence indicates that it was abundant in northern Mexico in the spring, that it spread and multiplied northward, and thus was aided by the wind in reestablishing itself. Moreover, races 21, 34, 36, 11 and 49 were very prevalent during one or more years since 1920 but have been found very rarely, if at all, since 1938. Although the wind is the only important agent of dissemination of inoculum of these races and is therefore extremely important in extending their geographic range, it cannot do the whole job alone.

And so the wind sometimes enables physiologic races to attain eminence from humble beginnings. But the existence of numerous races introduces still another aspect to aerobiology. In many cases the important question is not so much, how many spores are blown into a region by the wind, as what kinds of spores they are. As an example, there now are many varieties of spring wheats that are resistant to many, but not all, rust races (43). Unless these varieties have certain characters that tend to protect them against most races, the extent to which they will become infected during a spore shower depends on the proportion of spores that can infect them. Likewise, certain varieties of oats, notably Vicland, Tama and Boone, are resistant to some races of *Puccinia graminis avenae* and completely susceptible to others (133). The relative numbers of spores of the different races during a spore shower then determines the degree to which these varieties will become infected. And the test must be the actual development of rust on the varieties, not the total numbers of spores caught on spore traps, for the spores of the different races are morphologically indistinguishable.

The difficulties of effective aerial dissemination have been emphasized because things are not always as simple as they seem. There is great variation in seasons and geographic areas. Each problem must be studied thoroughly if all is to be learned that needs to be known. What spores will do to crop varieties when they reach them is the final and critical question, and the answer is not always easy to find.

SUMMARY

1. Although the possibility of aerial dissemination of certain plant pathogens was recognised by several workers a century or more ago, only during the last few decades have critical studies shown the importance of air-borne inoculum in the initiation of economically important plant diseases.

2. The wind may be of importance occasionally in the dissemination of certain bactérial plant pathogens and in the dissemination of viruliferus insects, or even of certain viruses themselves. The most important and heaviest load of plant pathogenic inoculum carried by the wind, however, is that of fungus spores, since many of the fungi which cause plant disease depend almost exclusively upon the wind for regular dispersal of their spores.

3. Most of the plant pathogenic fungi disseminated by the wind produce enormous numbers of spores in a minimum of time and space; usually these spores are forcibly expelled or abjected into the air, often during the time when optimum conditions for dispersal or infection prevail.

4. Most of the fungus spores disseminated by the wind are exceedingly buoyant, and can easily be caught by even minor convection currents and quickly carried to heights of several miles. They have been trapped as high as 36,000 feet, and many of them are present in abundance one to two miles above the earth. Because they fall at a rate that is measured only in millimeters per minute in still air, they are capable of being carried hundreds or even thousands of miles by the wind.

5. Mere distribution or dissemination of the spores of plant pathogenic fungi by the wind must be distinguished from effective distribution. Effective dissemination depends upon viable spores being carried to a suitable infection court of a susceptible plant at the proper stage of development and under conditions which will permit infection. This involves not only survival of the fungus inoculum during the journey by air, but also certain factors of timing in relation to weather and crop development. From the standpoint of the fungus, effective dissemination means also the dissemination of races able to attack the varieties of plants upon which they are deposited.

6. The spores of some fungi, such as the conidia of *Phytophthora infestans* and *Sclerospora philippinensis* and the basidiospores of *Cronartium ribicola, Puccinia graminis* and many other rusts, are produced only during periods of high humidity, and can survive only a short period of desiccation or exposure to sunlight. This, plus the fact that spores fall much more rapidly in moist air than in dry air, tends to limit effective dissemination of such spores to comparatively short distances. Spores such as the conidia of *Helminthosporium, Alternaria, Penicillium,* the chlamydospores of most smuts, and the aeciospores and urediospores of practically all rusts may be produced in humid atmosphere, but are liberated in dry air and survive long-distance travel in dry air.

7. Effective short-distance dissemination of many plant pathogens by air has been well established, among them being *Phytophthora infestans, Sclerospora* spp., *Venturia inaequalis, Endothia parasitica, Hypoxylon pruinatum* and many of the rusts and smuts.

8. *Puccinia graminis tritici* furnishes one of the best examples of regular, successful, long-distance dissemination of an important plant pathogen. Studies carried on for more than 20 years have shown that in the area bounded by the Rocky Mountains on the west and the Mississippi River on the east, this fungus has been able to maintain itself as a constant menace to wheat by urediospores blown northward in the spring and early summer, and southward in late summer and fall. Although the course of development of stem rust will vary from year to year, and from region to region in any one year, depending upon the interplay of many factors, the fact that air-borne urediospores initiate infection and that they are carried from Texas or north Mexico northward in the spring and

summer, and from Canada or the northern States southward in the fall, is too well established to be questioned.

It has been almost equally well established that epidemics of stem rust in the east central States are often initiated by primary infections from aeciospores borne on barberries in Virginia and neighboring States.

Further evidence, if it were needed, of the importance of air currents in the regional spread of wheat stem rust is furnished by the fact that areas such as southern Mexico and the Palouse district of western Washington, both of which are separated from the Great Plains area by formidable barriers, are more or less a law unto themselves. There is no regular exchange of rust between those areas and the Great Plains.

Similar seasonal spread of *P. graminis tritici* with regular seasonal winds has been observed in Canada, Australia, India and several countries of Europe, and, although the evidence in all cases is not conclusive, it is sufficiently strong to warrant the conclusion that air-borne spores play a large rôle in rust epidemiology in those countries also.

Similar interregional dissemination has been proven for leaf rust of wheat, stem rust of oats and crown rust of oats.

9. Even where a disease, as such, may persist in a certain region from year to year independently of wind-borne spores from another region, as late blight of potatoes in New England or wheat stem rust in some of the northern States where barberries still are present, interregional wind dissemination of certain virulent physiologic races may still play a very important part in epidemiology. More precise evidence of this has been accumulated with certain of the cereal rusts than with other plant pathogens, but the principle very probably applies to other plant pathogens also.

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THE BOTANICAL REVIEW

VOL. XII MAY, 1946 No. 5

CYTOLOGY OF CEREALS. II*

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INTRODUCTION

The first decade, freely speaking, of intensive work in the cytology of "small grain" cereals (1), and closely related grasses, may be characterized mainly by determination of chromosome numbers in supposedly well established races and species, and by description of general chromosome behavior in a number of varietal, specific and generic hybrids. The second, or immediately past, decade, and the sundry burden of this review, has become progressively more marked by increased artificial induction of disturbances of the apparently stable state of nature, or perhaps, more exactly speaking, by attempts to speed up processes of slowly rolling spontaneous evolution. A rugged stability on one hand is yet highly susceptible to change which often manifests itself cytologically as chromosome aberrations, numerical and structural.

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* Supplement to article in The Botanical Review 1: 467–496. 1935.

EUPLOID CHROMOSOME ABERRATIONS

Spontaneous Autoploid Aberrations

The occurrence in plant species of natural races with unlike chromosome numbers is no longer considered rare. Temporary confusion, sometimes resulting from diverse taxonomic interpretations, demands permanent recorded data and ample plant specimens to assure the future validity of the respective cytological research. These precautions will become even more imperative as the number of autoploid and alloploid aberrants are multiplied. Avoidance of further confusion in nomenclature merits a close cooperation of both cyto-geneticists and taxonomists in naming newly obtained aberrants, spontaneous or induced, that may become destined to exist as permanent species.

The evidence for spontaneous euploid duplication or reduction may seem fairly conclusive to quite obscure. Müntzing (343) has appropriately stated that all alloploids are partially autoploid. Most of the forms of the newly discovered Georgian wheat species, *Triticum Macha* Dek. et Men. (330), possessed the somatic chromosome number 42, but a small group had the number 28. Morphologically the hexaploids and tetraploids were almost identical. The tetraploids were thought to have arisen from the hexaploids by mutation, and have been designated as *Triticum dicoccum* ssp. *georgicum* Dek. et Men. Tumanian (534) found in the progeny of a wild diploid wheat, in addition to tetraploid individuals, a tetraploid head on a diploid plant. The mutant head generally, though not completely, resembled in enlarged dimensions the original form, and was named *Triticum Jerevani.* Robertson and Weaver (433) found a giant, apparently tetraploid, form of *Agropyron spicatum* growing adjacent to the normal diploid.

When races of unlike euploidy in a species occur in diverse geographical regions, the specific bond is assumed usually on the strength of morphological likeness. *Agropyron junceum* occurs as a tetraploid along the Atlantic shores from Portugal to Scandinavia, and is present as a more robust hexaploid along the shores of the Mediterranean (385). *Agropyron cristatum* comprises diploid, tetraploid and hexaploid races (32); *A. intermedium,* tetraploid (209) and hexaploid (32, 209); *Hordeum nodosum,* diploid and tetraploid (82, 510); and *H. murinum,* diploid (393, 528) and tetraploid (30, 82, 529). Summaries of chromosome numbers (528, 530) will reveal yet other species, including races or subspecies, with dissimilar euploidy.

Decrease in euploidy is most commonly observed in the form of haploidy. Haploids arise spontaneously in plant populations at frequencies apparently determined by species, race and ecological conditions. Raw (429) attributed the 0.0001% frequency of haploids in *Triticum vulgare* to excessively high temperature at time of flowering. Yamasaki (571) found a frequency of 0.029% of wheat haploids among 33,600 non-hybrids, and 0.025% among 211,600 hybrids. Volunteer haploids have been found also in *T. monococcum* (495), *T. spelta* (484) and *Secale cereale* (285).

One source of spontaneous euploid chromosome aberrants is polyembryony. Yamamoto (570) has found that the incidence of polyembryony is variable with plant, strain or hybrid, and he is, no doubt, justified in assuming that twinning is genetically conditioned. Tabulated reports by Müntzing (346) corroborate this assumption; *e.g.,* the "Stålråg" variety of rye produced a much higher proportion of multiple embryos than the "Kungsråg". An approximate summary of available counts of multiple embryos in cereals, including *Triticum* (257, 346, 568, 569), *Secale cereale* (257, 346, 460), *Hordeum vulgare* (346) and *Avena sativa* (346), reveals that 2,186,486 caryopses produced 764 sets of twins, seven sets of triplets and one set of quadruplets, or, briefly, one set of multiple embryos per 2,832 grains. In *T. monococcum* twin embryos occurred only after cross pollination, and with increased frequency following delay in pollination (221), attaining under the latter conditions a high frequency of three pairs of twins among 195 seedlings.

Table 1, based on most of the compilable data (213, 257, 346, 347, 460, 568, 569), shows that polyembryony in cereals is a poor source

TABLE 1

RATE OF OCCURRENCE OF ABERRANT CHROMOSOME NUMBERS AMONG MULTIPLE EMBRYOS

Plant species and 2*n* chromosome No.	No. of caryopses	No. of embryos and degree of euploidy		
		½ × 2*n*	1½ × 2*n*	2 × 2*n*
Triticum vulgare (42)	246	4 (21)	23 (63)	0
T. durum (28)	8	1 (14)	0	0
T. turgidum (28)	3	0	2 (35)	0
Secale cereale (14) .	799	2 (7)	2 (21)	1 (28)
Hordeum vulgare (14)	47	2 (7)	1 (21)	0
Avena sativa (42) .	131	0	23 (63)	1 (84)
Total	1234*	9	51	2

* Includes six sets of triplets and one set of quadruplets, occurring mainly in *Secale*.

of "tetraploids"[1], a fair source of haploids and a comparatively rich source of "triploids". Among 1,234 polyembryonal caryopses, approximately 60 produced a pair of twins with unlike chromosome numbers. A *Triticum vulgare* triplet produced two "triploids" and one normal (569).

The origin of multiple embryos, in general, has been fully discussed by Webber (566). The source of the second member of a "diploid" pair of twins has been attributed to post-fertilization cleavage (419) and to antipodal fertilization (257); the "triploid" member of a "diploid-triploid" pair to embryonal development of a fertilized endosperm cell (257, 569) and to a fertilized unreduced nucleus of a supernumerary embryo sac (346); and the haploid member of the less frequently occurring haploid-"diploid" combination to parthenogenesis of the reduced egg cell (221, 569). The fortunate finding of twins in F_1 hybrids, as reported by Kasparyan (190), reveals more definitely to what extent the ♂ elements participated in the formation of the embryo. In a varietal tetraploid wheat cross, one twin was a normal "diploid" hybrid, the other a haploid replica of the ♀ parent and must have sprung apomictically from an extra egg cell, antipodal or synergid. A hexaploid-tetraploid wheat cross proved even more instructive in that one twin was a hybrid with the expected three genoms from the ♀ and two genoms from the ♂; the second twin also was a hybrid with three genoms from the ♀ but apparently with four genoms from the ♂, indicating, perhaps, that one pair of sperms fertilized the egg and endosperm, and a second pair fertilized an extra egg cell, a synergid, or an antipodal.

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[1] Tetraploid, triploid, etc., in quotation marks are used in the loose sense in this review to signify 2× and 1½× somatic number, respectively, and may or may not correspond to the number of genoms.

Outside the category of polyembryony the exact causes of spontaneous euploid chromosome changes are equally obscure. Some are no doubt the result of mitotic aberrations in vegetative tissue in a cell line leading to sex cells (285), and may include larger portions of the plant, demonstrated by various forms of chimera (357, 534), or may be discovered as small blocks of aberrant cells in reproductive tissue, as entire locules, or parts of locules of anthers (78, 276, 344, 577). Large spore mother cells, with multiplied euploidy, either singly or in smaller or larger groups, are not an unfamiliar sight in routine studies of anther material. An extreme case of multiploid sporocytes was observed in *Hordeum vulgare* (502). As hybrid material is perhaps more intensively studied and also more unstable, reports (107, 110, 224) have been more numerous from this source. Drought (224) and high temperature (177) are apparently conducive to development of these large cells.

Haploid cells in unreduced tissue in a "diploid" plant are rare. Haploid pollen mother cells were, however, observed in a 42-chromosome segregate of a pentaploid wheat cross (296).

Induced Autoploid Aberrations

"Tetraploids" have developed after treatment with colchicine in wheat (100, 392), rye (57, 85, 461), barley (78, 100, 145, 188) and oats (100, 461); after exposure to X-rays in rye (56); and after exposure to high temperatures in wheat (99), rye (99) and barley (121, 185, 186, 358).

One triploid plant of *T. monococcum* was obtained after pollination with X-rayed pollen (230). Also one triploid and one short-lived octoploid were obtained in rye through colchicine treatment (57).

Haploids have arisen in rye following exposure to high (375) and low (345) temperatures. Emasculation with subsequently attempted hybridization has resulted in haploids in wheat (362, 495, 557), and in conjunction with colchicine in *Aegilops ovata* (325). Emasculation by exposing the green immature anthers to drying atmosphere raised the frequency of haploids, as well as of hybrids, in common wheat (266). X-raying of the pollen previously to its application to the emasculated florets seemed to favor the production of wheat haploids (192, 194, 230, 578), increasing the production of haploids in a strain of *T. monococcum* from 0.5% to 13.7% (221). That the pollen, though not fertilizing, may in some way energize the egg to parthenogenetic development was further illustrated by delayed pollination with foreign pollen (221, 222). Emasculated florets of *T. monococcum* were pollinated with pollen from another diploid, *T. aegilopoides,* or with hybrid pollen from the cross of the two wheats. Pollination six days after emasculation gave rise to 20% of haploids, nine days to 37.5%, and five days to none, while emasculation alone produced no seed. Microscopic examination revealed that the egg cell may develop parthenogenetically into a small haploid embryo, and Kihara (221) was of the opinion that further development would cease unless fusion of a sperm nucleus with the polar nuclei arouses endosperm development.

The degree and sometimes type of morphological and physiological response to autoploid chromosome duplication vary with genus, species and even with the variety (188) and race affected. Auto-"tetraploids" of *Haynaldia villosa* (232), species of *Aegilops* and *Secale cereale* (85, 232) presented a *gigas* habit.

A commonly used criterion for degree of polyploidy is stomatal size. Tetraploids of diploids in *Hordeum* (78, 103, 121, 145), *Secale cereale* (56, 57, 85, 232), *Haynaldia villosa* and species of *Aegilops* (232) showed definite increase over diploids in size of guard cells, an increase in some cases approaching 30%. The large size of epidermal cells causes a correspondingly greater dispersal of the stomata. Breslavetz (57) found the cell and nuclear size in rootlets of autotetraploid *Secale cereale* larger than in the corresponding diploid, triploid and octoploid. Triploid cells ranked second in size. The stomata of the auto-"triploid" *Triticum vulgare* were larger than those of its "diploid" (569). Pollen size also was found to be larger in auto-"tetraploids" (85, 121, 145, 186, 232) and auto-"triploids" (277).

Tabulated comparisons of the diploids and tetraploids of cultivated barleys (78, 121, 145, 185, 186, 188) indicate that all currently desirable qualities may not be in favor of the "tetraploid". In general, the "tetraploids" had somewhat shorter and thicker stems; larger, broader and thicker leaves which at least in some instances were deeper green (78, 188); and possibly in some cases, possessing slightly larger chloroplasts (121). Kostoff (258, 261), in studying the effect of altered euploidy in some members of the Gramineae and Solanaceae, arrived at the conclusion that chloroplast size is independent of chromosome number. Levan (286) found in representatives from 11 plant genera that chlorophyll content per fresh weight in polyploids is usually lower than in diploids and due in part to greater leaf thickness in polyploids. The number of stems in tetraploid barley was apparently variable with race concerned and growth conditions. The rachis was frequently longer but bore fewer flowers which, at least in some races, were larger (188). The number of mature caryopses per rachis was reduced, not only by the smaller number of flowers present but also by the failure of some of the flowers to set fruit. The number of abortive flowers was seemingly determined by plant race (185, 186, 188) and ecological conditions. The total fertility of tetraploids was further diminished by lowered germinability (121, 145, 352). The reduced fertility in barley was in part offset by the larger size and greater weight of grain (78, 121, 186, 188, 352). This grain size relationship held true also in *Avena brevis* (119) and *Secale cereale* (57).

Tetraploidy did not affect the normal self sterility inherent in diploid rye (57). Sengbusch (461), by propagating larger blocks of the tetraploid rye, obtained an increase in yield from 5% or less to 40%.

Flowering of the "tetraploids" was reported as delayed (78, 119) or as simultaneous with diploid, but with prolongation of the open flower period (188). Seed germination was retarded two days or more (78, 121, 145).

Other physiological comparisons recently made in barleys, though not necessarily in agreement (29, 79, 80, 103, 121, 145), point to marked differences between the tetraploid and its corresponding diploid under a given set of conditions. Thus respiration rate was found to be lower (79), while malt diastase activity was twice as great in germinating tetraploid grains as in the corresponding diploid, and catalase activity of the seed powder was two and a half times greater in the tetraploid grains (80). Meagre chemical analyses (79, 103, 145, 352) point to higher contents of proteins (79, 352), ash (79, 103, 145) and sometimes sugars (103) in the tetraploids.

Reactions of autopolyploid plants to external agents and conditions may become significant in interpreting relative survival values of polyploids in nature. Diploid and autotetraploid grains of rye and barley displayed about an equal degree of resistance to high temperature, but the tetraploid survived X-ray treatments with less injury (503). Tetraploid barley survived the X-ray dosages with better germination, vigor and fertility of the resulting plants (350). A number of chlorophyll deficiencies present in the X_2 generation of the diploid were absent from the tetraploid (353).

It is known that the auto-"tetraploid" may display a reaction of its own in hybridization. Autotetraploid *Avena brevis* pollinated by its diploid set no seed; pollinated by *A. sativa* ($2n = 42$) or by *A. barbata* ($2n = 28$) it set seed readily, though diploid *A. brevis* failed to cross with these two species (119). *Secale cereale* autotetraploid ♀ × diploid ♂ produced seed, but diploid ♀ × autotetraploid ♂ was sterile, due to failure of pollen growth (85). *Triticum vulgare* auto-"triploid" ♀ × diploid ♂ set 68.8% of grain, but diploid ♀ × auto-"triploid" ♂ set no grain (569). "Tetraploid" heads of an *Aegilops ovata* chimera, 26.47% fertile when artificially selfed, were 25% fertile when pollinated by "diploid" heads of the same plant (325). However, the "diploid" heads pollinated by the "tetraploid" were only 15.9% fertile.

Karpechenko (188), in enumerating the defects of autotetraploid barleys, mentions the occasional susceptibility to ergot, which was particularly high in one variety.

In meiosis of auto-"tetraploids", bivalents and quadrivalents seemingly vie for supremacy, the preeminence of one or the other being determined evidently by the particular genetic complex involved (78) and, no doubt, also by conditions external to the plant. Autotetraploid *Secale cereale* (85, 232), including the $4n$ part of a chimera (357), *viz., Haynaldia villosa* and species of *Aegilops* (232), produced a mean of one or two quadrivalents, though a maximum of four or five was observed in some forms. Autotetraploid *Hordeum* sometimes displayed the possible maximum of seven quadrivalents with two or three as the mean (121, 401). Auto-"tetraploid" *Aegilops ovata* with 56 somatic chromosomes produced both quadrivalents and bivalents (325). The naturally occurring tetraploid race of *Agropyron cristatum* formed a mean of 3.7% quadrivalents (359); and if quadrivalents are a criterion for autotetraploidy, both this race of *A. cristatum* and the natural species, *Hordeum bulbosum* with a maximum of seven and a mean of four or five quadrivalents (45, 83), are probably true autotetraploids, each with four identical genoms. Autotetraploid meiosis was often characterized by bridges, higher autosyndetic associations and other indications of structural chromosome changes (78, 379); furthermore, univalents were not always absent. Müntzing (357) observed a lower chiasma frequency in 4*n* than in 2*n* spikes of a *Secale cereale* chimera.

Auto-"triploids" of known origin exhibited also a strong tendency to form trivalents, autotriploids *Triticum monococcum* (230) and *Secale cereale* (257, 277) forming up to four and five or six, respectively. Yamamoto (569) found in the auto-"triploid" *Triticum vulgare* with 63 chromosomes many trivalents of various constructions, but rarely the possible maximum number of 21.

The life expectancy of non-perennial cereal auto-"triploids", characterized as they are by meiotic irregularity in chromosome distribution, is short. Auto-"tetraploids" are usually comparatively stable, but all forms cannot be depended on to breed true. If open pollinated, though only weakly amenable to back-crossing to the corresponding diploid, "tetraploids" may give rise to "triploids" which segregate into diploids and aneuploids. Exclusion of 1*n* pollen did not entirely prevent production of aneuploids in *Secale cereale* (354). In 149 lines of offspring of autotetraploid barley, Chen (78) observed among forms with cytological aberrations, diploids with reduced fertility, hypo- and hypertetraploids, tetraploids with 2*n* and 4*n* pollen in the same anther, and tetraploids with various types of meiotic irregularities. Lines of tetraploids with 14 bivalents had rather high fertility compared with the other groups.

Haploids, according to tables presented by Modilevski (337), now are known in 44 genera representing many families. Haploidy in angiosperms is discussed also by Ivanov (166).

The haploid in cereals is usually a less robust replica of its diploid parent, and has in some cases been recognized by smaller and less dispersed stomata (568). According to Yefeikin and Vasiliev (557, 578), haploids of the durum or tetraploid group of wheat, as *T. persicum* and *T. dicoccum,* bore striking resemblances to wheats of the einkorn or diploid group in respect to head characters and conspicuous nodal pubescence. It may be relevant in relation to these results that Camara (67), in reviewing the interrelationships of wheat, noted that some wild diploid species, especially *T. Thaoudar,* show very close resemblance to *T. dicoccoides* and others of the tetraploid series. The latter author suggested that tetraploid species of wheat may have arisen from diploid by chromosome duplication followed by chromosome changes. Having observed the results of X-rays, he thought it possible that through simple processes of evolution, as fragmentation, translocation and other structural changes, there could be constructed from the chromosomes of the diploid the complete series of chromosomes of the tetraploid.

The usual great preponderance of univalents in the first meiotic division in haploids has been further confirmed in the diploid species *Triticum monococcum* (230), *Hordeum distichum* (531) and *Secale cereale* (285, 375); in the tetraploid species *A. ovata* (325) and *T. durum* (213); and in the hexaploid species *T. vulgare* (257, 274, 288, 429, 568, 572). The approximate average, based on calculable reports, of pollen mother cells with univalents exclusively is 80% in diploid and tetraploid species, and drops to 60% in the hexaploid *T. vulgare,* indicating, perhaps, inclusion in the hexaploid of more chromosomes with partial homology. However, there are various other factors that apparently govern pairing in the haploid. Some of these are external; others, including genetic, are internal. Levan (285), in a comparative study of three haploid rye individuals, observed that pairing varied from plant to plant, and in one instance from locule to locule. Two of the haploid individuals presented ten times the number of chiasmata of the third.

Haploids, in cereals, like triploids are cytologically highly unstable and perish after flowering, or revert to "diploids" through formation of restitution nuclei in the germ cells. The reverted "diploids", according to Kostoff (266), may not, though selfed, be heterozygous, as crossing-over between autosyndetically conjugated chromosomes may lead to structural chromosome changes. Segregation of morphological and physiological characters occurred in reverted "diploids" of *T. vulgare.* Sears (450, 451, 455, 457) found that the progeny of haploid *T. vulgare,* when pollinated by the "diploid", produced an excellent source of cytological aberrations, including nullisomics and polysomics. Some of the aberrations were accompanied by definite phenotypic effects. Use of chromosome aberrants in "diploids" derived from haploids has proved to be a successful method of analyzing *T. vulgare,* and Sears suggests that other supposedly polyploid plants may be amenable to this same sort of analysis.

Undoubled Interspecific Hybrids

Data on cytology of hybrids has been augmented markedly and especially in the field of amphidiploidy. A brief summary, covering reports to 1937, on chromosome pairing in specific and generic cereal hybrids has been compiled into a serviceable 21-page table (214). Other summaries covering also some more recent work are available (86, 149, 265).

Cytology of interspecific hybrids in *Triticum* covers crosses within a chromosome group, as diploids (496), tetraploids (31, 39, 74, 84, 86, 87, 234, 238, 243, 307, 516) and hexaploids (88, 105); and crosses between chromosome groups, as diploid x tetraploid (31, 238, 239) and tetraploid x hexaploid (49, 86–88, 106, 147, 160, 168, 238, 302, 390). Some of these hybrids involve, besides the hexaploid *T. Macha* (88), two newly discovered Chinese tetraploids, "blue" wheat and dwarf hill-wheat from Szechwan (87). Of the hexaploid crosses, *T. Macha* x *T. vulgare* was weak and failed to form pollen mother cells (88), and *T. Macha* x *T. spelta* was 63.5% sterile and at meiosis produced 13–21 bivalents. Univalents, rings of four or six or chains of three or five chromosomes, and bridges were present. Apparently *T. Macha* differs somewhat both cytologically and genetically from other known hexaploid wheats. *T. Macha* x tetraploid wheats differed little from other pentaploid crosses except the cross involving *T. dicoccum* as the second parent which was almost sterile due to crumbling of chromosomes at the end of the second division.

The hoped-for goal of combining the most desirable agricultural qualities of hexaploid wheats with the high disease resistance of some tetraploid wheats has led to the continuance of extensive work on pentaploid wheat offspring (144, 147, 217, 226, 229, 298, 299, 301, 303, 310–316, 323, 324, 327, 384, 398, 442, 443, 462, 521, 575, 576), including back-crosses (37, 317–321, 324, 462, 575).

Cytological data on interspecific hybrids in *Aegilops* have with some exceptions (46, 292, 454, 505) been augmented by Kihara and his collaborators (167, 214–216, 219, 225, 227), and have been summarized (214) and discussed by Kihara in relation to a new classification scheme (218, 220, 223) based in part on cytogenetical results. The genus is divided into six sections, and each section, unlike in *Triticum,* may contain more than one chromosome group. The species in a section are thought to have at least one genom in common.

Secale cereale × wild *Secale* species had seven bivalents and showed high fertility, except *S. secale* × *S. montanum* which had five to seven bivalents (245) and some other chromosome irregularities, and suffered consequent reduction in pollen fertility (102). Kostoff (245) pointed out the value of *S. ancestrale* as a robust and productive species worthy of trial in a hybrid with *S. cereale.*

Interspecific hybrids in *Hordeum* have recently been reported (427).

Little is known as to the cytogenetical relationships within the comparative large genus *Agropyron.* Cytology has been described for apparent hybrids, of spontaneous origin from five localities in Sweden, between tetraploid *A. junceum* and hexaploid *A. repens* (381, 383). Seven plants were pentaploid, as expected, and at meiosis had nine to 13 bivalents in addition to univalents. Plants from one locality were heptaploid, having arisen presumably through back-crossing of the pentaploid hybrid to *A. junceum,* or through fertilization of an unreduced *A. junceum* gamete by *A. repens.* Both hybrids were heterozygous for inversions, and both were highly sterile. Also other probable *Agropyron* hybrids of natural origin have been discovered (480, 481).

Some interspecific *Avena* hybrids within the diploid and tetraploid chromosome groups produced, besides bivalents, a ring of four chromosomes, explained on the basis of segmental interchange (108, 110). Diploid × tetraploid formed five to seven pairs (109, 368, 464) and showed decreased fertility. Chromosome morphology (464) and general cytogenetic characteristics point, however, to a close relationship between species of the diploid and tetraploid chromosome groups (111). Tetraploid *A.. barbata* (AAB′B′) × autotetraploid *A. strigosa* (AAAA) formed seven bivalents contributed by homologous genoms AA, and, in addition, five or six bivalents derived through pairing between partially homologous genoms A and B′ (370). The formation, on the average, of more than seven bivalents in diploid *A. longiglumis* × hexaploid *A. sativa* (109) also substantiates the incidence of autosyndesis. Comparatively low pairing and high sterility characterized pentaploid oat hybrids, as *A. fatua* and *A. barbata* (373). An octoploid plant with 28 bivalents arose among other F_4 progeny of the back-crossed pentaploid, but did not breed true.

Undoubled Intergeneric Hybrids

The majority of intergeneric crosses in small-grain cereals involve the genus *Triticum* as one parent.

Compared with *Aegilops cylindrica,* other *Aegilops* species showed apparently less chromosome homology with species of *Triticum,* though a variable number of bivalents, usually of the open formation, were present (195, 214, 390, 453, 454, 507). Tests made by Miège (333–335) suggested that the introduction of *Aegilops* into wheat may improve its baking quality. Species of *Aegilops* as well as *Haynaldia villosa* contained more mineral matter, nitrogen and gluten, and less starch than wheat.

The genoms of diploid *Haynaldia villosa* (214, 228, 238, 239, 453, 454) and *Secale cereale* (214, 290, 363) met little or no homology in crosses involving species of *Triticum.* The tetraploid *Haynaldia hordeacea* Hack., an extremely hardy and drought-resistant perennial, in serving as ♂ parent in a cross with Vjatka rye, gave rise to a fertile hybrid (598).

Among desirable characteristics (98, 147, 329, 434, 438) obtained in different wheat-rye hybrids and their segregates have been various types of disease resistance, cold and drought tolerance, early maturity and good grain quality.

Some species of *Agropyron* cross with *Triticum,* and apparently more successfully when *Triticum* is the ♀ parent. Cytology of *Triticum* × *Agropyron* is known in hybrids including tetraploid and hexaploid chromosome groups of *Triticum;* and four species of *Agropyron,* namely, the tetraploid and hexaploid races of *A. intermedium* (syn. *glaucum*) (35, 208, 209, 400, 405, 424, 488, 535, 541, 556), the tetraploid race of *A. junceum* (382), the hexaploid *A. trichophorum* (305) and the decaploid *A. elongatum* (208, 209, 400, 404, 405, 423, 424, 540, 548–551, 553, 555). Variations in meiotic chromosome configuration occurred in all combinations, indicating incomplete homology between *Triticum* and *Agropyron* genoms, and also a difference in the response of ecotypes and races entering into a particular cross. The maximum number of possible bivalents was approached more often in the F_1 with the highest chromosome number. Autosyndesis between two *A. elongatum* genoms was apparently responsible for the large number of bivalents when this decaploid species entered the cross (209, 400, 548, 549). Assumption of the quadruple state of one genom of *A. elongatum* seems to be substantiated by the finding of an average of three quadrivalents and frequently a ring of eight chromosomes in the spore mother cells of a race of this species (400). Other high chromosome aggregates, in addition to about 22 bivalents and two univalents, were also found.

Irregularities in meiosis of the hexaploid *A. intermedium* (syn. *glaucum*) suggest previous interchange of segments between nonhomologous chromosomes. The tetraploid race of *A. intermedium* apparently lacks at least one type of genom found in the hexaploid race (400, 548). The phenotypic effects of the exclusion or inclusion of a genom has evidently not been of such magnitude as to give the two races separate specific rank.

Two F_1 plants of *T. durum* ($2n = 28$) × *A. trichophorum* ($2n = 42$), though quite similar as to morphology and high rust resistance, were markedly divergent as to meiotic chromosome behavior (305). One plant had a large number of univalents and an average of 1.6 bivalents per sporocyte; the other plant had an average of 6.06 bivalents and frequently a chain of three chromosomes, and inversion bridges were present in 4% of the cells. [As little was known as to the chromosomal constitution of the two parents, it was impossible to decide whether the differences found in the two plants should be ascribed to strain differences in the parents, or to the functioning of antipairing genes in one of the plants.] *T. Macha* ($2n = 42$) × *T. trichophorum* ($2n = 42$) produced an average of 7.7 bivalents per sporocyte and frequently chains of three or four chromosomes. Inversion bridges were present in 25% of the cells. The fertility of all the hybrids involving *A. trichophorum* was extremely low. Crosses are reported to have been obtained also between *Triticum* species and *A. obtusiuscum* (*sic*) (270).

Fertility in *Triticum-Agropyron* crosses has been variously described as low to comparatively high, and has often bettered with succeeding generations. The agronomic values apparently depend on races of *Triticum* and *Agropyron* entering the cross. Some of the more meritorious characteristics observed, singly or in various combinations (89, 143, 430*a*, 439, 459, 465, 479, 492, 536, 538, 539, 541, 543, 561–563), are high protein content of grains, superior baking qualities, large grains, high grain yield, standing capacity, early maturity, drought and saline tolerance, and rust and smut resistance. Some segregates are annual, others perennial. Some may produce a good hay crop in addition to the grain harvest. The *Triticum-Agropyron* cross, like other intergeneric crosses, is still in the early experimental stage.

Hybrids between *Triticum vulgare* ($2n = 21$) and *Elymus arenarius* ($2n = 56$) were obtained when each parent plant had developed from an embryo grafted onto the endosperm of the foreign genus (411).

Meiosis in the diploid hybrid *Haynaldia villosa* × *Aegilops* indicated only little chromosome homology (46, 214) between the two genoms.

The hexaploid race of *Agropyron intermedium* in crosses with diploid, tetraploid and hexaploid species of *Aegilops* produced numbers of bivalents approaching the possible maximum (116), and is cytologically as close, it seems, to these *Aegilops* species as it is to *Triticum.*

Secale cereale, in crosses with tetraploid species of *Aegilops* (214) and the hexaploid race of *Agropyron* (540), presented a higher bivalent formation than in crosses with species of *Triticum* with which it apparently has no chromosomes in common. Crosses have been obtained also between *Secale* and the following species of *Agropyron: repens, sibiricum, trichophorum* (491) and *cristatum* (93, 272).

Hordeum distichon var. *nutans* Schübl. ($2n = 14$) ♀ × *Elymus giganteus* ($2n = 28$) ♂ produced a hybrid plant with 21 chromosomes (39*a*).

Back-crossing in intergeneric hybrids, as *Triticum* × *Secale* (120, 196, 197, 200, 202, 281, 533) and *Triticum* × *Agropyron* (208, 211, 405, 533), resulted in various chromosome combinations and sometimes in sesquidiploids (208, 209, 211) and amphidiploids (120).

Use of later generations of hybrids to pollinate F_1 hybrids (380, 541) has in some instances been preferable to back-crossing to improve fertility as well as to bring about favorable segregates.

Triticum × *Secale* progeny (196, 197, 199, 200, 202, 203, 281) has served in studies of genom stability.

Interspecific Amphidiploids

Amphidiploidy, arising, generally speaking, through recovery of genom "diploidy" from aberrant alloploid genom haploidy, has no doubt played an important rôle in the building of natural species; and increased ingenuity in the use of polyploidogenic agencies will greatly multiply the number of amphidiploids in selected genera.

Triticum crosses elevated from triploids to hexaploids through amphidiploidy are: *T. durum* ($2n = 28$) × *T. monococcum* ($2n = 14$) (581, 583, 585, 591, 595), named *T. Edwardi* Zheb. (594); *T. Timopheevi* ($2n = 28$) × *T. monococcum* ($2n = 14$), named *T. Timococcum* (236, 254); and *T. persicum* ($2n = 28$) × *T. monococcum* ($2n = 14$) (191). Amphidiploids of the octoploid series are: *T. polonicum* × *T. durum* (584); *T. Timopheevi* ($2n = 28$) × 28-chromosome wheats of different species and varieties (582, 583, 586, 589–595), all named *T. soveticum* Zheb., the 28-chromosome parent entering the cross with *Timopheevi* supplying the subspecies name, *e.g.*, *T. soveticum* ssp. *durum* (589, 594). Amphidiploid *T. Timopheevi* × *T. persicum,* included in above group, has been designated as *T. fungicidum* by another author (597, 598) to signify its high resistance to fungal diseases. The amphidiploids in this octoploid series involving *T. Timopheevi* as one parent are generally characterized by high disease resistance and very large grain (582, 596, 597). An exception in respect to disease resistance is *T. Timopheevi* × *T. orientale* which derives a susceptibility to mildew from *T. orientale* (592), a species of low resistance to mildew and rust, low adaptability and limited distribution. The different hybrids of the octoploid series intercross readily (594). In the decaploid series are amphidiploids *T. Timopheevi* ($2n = 28$) × *T. vulgare* ($2n = 42$) (587, 591, 595), named *T. Borisovi* Zheb. (594), and *T. durum* ($2n = 28$) × *T. vulgare* ($2n = 42$). A dodecaploid amphidiploid *T. vulgare* ($2n = 42$) × *T. compactum* ($2n = 42$) has been reported (99). *Triticum* amphidiploids, like amphidiploids in general, present in varying degrees a less regular meiosis and lower fertility when compared with well established species.

In the genus *Aegilops* hexaploid (220) and numerous tetraploid (216, 232, 328, 449, 453, 454) amphidiploids have been developed.

Amphidiploid *Ae. caudata* ($2n = 14$) × *Ae. squarrosa* ($2n = 14$) has been described as a plant similar to *Ae. cylindrica* ($2n = 28$) in respect to morphology and chromosome constitution (328). *Ae. cylindrica* is thought to contain the C genom in common with hexaploid wheats, and possibly also in common with *Ae. squarrosa,* and to contain a second genom in common with *Ae. caudata.*

Intergeneric Amphidiploids

The first cereal amphidiploids cytologically studied were all intergeneric, as *Secalotricum* (or *"Triticale"*), *Aegilotricum* and *"Haynaldtricum"*.

A hexaploid amphidiploid, *Triticum durum* ($2n = 28$) × *Secale montanum* ($2n = 14$) (97, 98), has been said to be a vigorous, drought-tolerant, disease-resistant and high grain-yielding perennial. Octoploids of the *Secalotricum* type, amphidiploid *T. vulgare* ($2n = 42$) × *S. cereale* ($2n = 14$) (47, 99, 147, 159, 344, 364, 552) have been produced, one by double back-crossing [(*T. vulgare* × *S. cereale*) F_1 × *T. vulgare*] F_1 × *S. cereale,* and apparently the result of the fertilization of an unreduced egg at each of the last two steps (120).

Müntzing (349), in a comparative study of six strains of *Triticum-Secale* amphidiploids from diverse sources, found that the strains differed from one another as to vigor, fertility, meiotic stability and general physiological and chemical aspects, and that they intercrossed with greater difficulty than different varieties of wheat. The direction of the cross was sometimes a deciding factor. Ease of self-fertilization is apparently inherited from the *Triticum* parent. Chemical analyses (273, 349, 438) disclosed that *Secalotricum* differed from the corresponding undoubled hybrid as to grain qualities. The future agronomic worth of the wheat-rye amphidiploid seems subject to diverse opinions. Müntzing (352) regards present types an improvement over the old.

Amphidiploids of *Triticum* × *Aegilops* have been developed between several chromosome series of the two genera. Sears (453, 454) has made a more extensive study of the tetraploid group. From cross to cross the average number of multivalents and univalents varied from 0.24 and 0.12, respectively, per sporocyte to 4.72 and 1.60, and with this increase in mitotic irregularity the corresponding percentage of non-abortive pollen dropped from 93.5 to

76, and the grain set dropped from 94 to 25.] *Aegilotricum* octoploids of various species combinations have been developed (376, 394, 458, 504, 506, 507). Amphidiploid *Ae. cylindrica* ($2n = 28$) × *T. turgidum* ($2n = 28$) in a cross with *vulgare* had 21 bivalents + seven univalents at meiosis, as should be expected if *Ae. cylindrica* and *T. vulgare* both possess the C genom. When *Ae. cylindrica* was replaced by *Ae. ventricosa,* homology seemed confined to fewer chromosomes, or to parts of chromosomes only (458). The frequent occurrence of trivalents and quadrivalents may possibly be explained by the behavior of *ventricosa* in a cross with another tetraploid wheat. The 42-chromosome sesquidiploid *Ae. ventricosa* ($n = 14$) × *T. dicoccum* ($14 + 14$) resulting from the back-cross to *T. dicoccum,* produced at meiosis 14 dicoccum bivalents and seven autosyndetic *ventricosa* bivalents (507). When the sesquidiploid was crossed with *T. vulgare,* usually 21 bivalents were formed and the fertility was 65%. With *T. spelta* as the hexaploid wheat parent the fertility rose to 86%.

The assumption that the C (or D = dinkel) genom of hexaploid wheats is of *Aegilops* origin has led to attempts to synthesize these wheats. The problem at present apparently is to locate a diploid species with the C genom either practically unaltered or altered along the pattern of the C genom of *Ae. cylindrica* and hexaploid wheats. Such an *Aegilops* species in an amphidiploid cross with most 28-chromosome wheats should be expected to give rise to a hexaploid plant generally similar morphologically and physiologically to the existing hexaploid wheats of natural origin. One such plant approaching the theoretical form, developed by Britten and Thompson (64, 524), was amphidiploid *T. durum* ($2n = 28$) × *Ae. speltoides* ($2n = 14$). It had the same chromosome number as *T. vulgare* and crossed readily with this species, without much consequent meiotic irregularity or lowered fertility. The amphidiploid differed from *T. vulgare* by a few minor morphological features, less meiotic regularity and lowered fertility. Doubt has been raised as to the possession of the C genom by *Ae. speltoides,* and A′ has been proposed as a substitute genom (436) on account of rather high bivalent formation in the undoubled hybrid and the presence of four nucleoli in *Ae. speltoides.* Four nucleoli have also been found in some diploid wheats which have been credited with the diploid genom formula AA. The above hybrid should therefore possess a maximum of eight nucleoli, whereas the *vulgare* wheats possess a maximum of six.

Another synthetic hexaploid, developed by McFadden and Sears (328), was amphidiploid *T. dicoccoides* ($2n = 28$) × *Ae. squarrosa* ($2n = 14$). This plant has been described as stable, highly fertile and morphologically almost identical with *T. spelta,* and in a cross with the spelt formed 21 bivalents in about half of the sporocytes, with multivalents rarely occurring. The performance of *Ae. squarrosa* in producing the synthesized copy of *T. spelta,* as well as that of *Ae. cylindrica* [see tetraploid *Aegilops* amphidiploids (328)], suggested to the above authors that *squarrosa* may carry the $2n$ genom formula CC.

McFadden (327*a*) has reported still other hexaploid amphidiploids in the *Triticum* ($2n = 28$) × *Aegilops* ($2n = 14$) group.

Naturally occurring *Aegilotricum* forms have been found in France (75), and apparently undoubled *Triticum-Aegilops* hybrids in wheat fields of Spain (158) and in other countries (101). Some forms of *Triticum-Aegilops* amphidiploids have been described as free from lodging and shedding (9).

Amphidiploid *Haynaldia villosa* ($2n = 14$) × *Triticum dicoccum* ($2n = 28$), named *Haynatricum* (598), is a self-pollinated annual with vitreous grains exceeding in length those of either parent.

The most sterile amphidiploid reported within the scope of this review is *Aegilops umbellulata* ($2n = 14$) × *Haynaldia villosa* ($2n = 14$) with no grain set, in spite of low pairing in the undoubled hybrid and few multivalents in the amphidiploid. More than half of the chromosomes in the amphidiploid meiosis remained unpaired (454).

Decaploid amphidiploids *Triticum* spp. ($2n = 28$) × *Agropyron intermedium* (syn. *glaucum*) ($2n = 42$ race) have been induced artificially, but the amphidiploid number in some of these crosses apparently readily arises spontaneously through selfing of unreduced gametes in the undoubled hybrid (157, 210). Meiotic regularity varies, possibly with races entering the cross, and growth locality. Bivalents ranged in number from 27 to 35 with a corresponding number of univalents and occasional multivalents (36, 157, 406). The plants have been described (157, 210, 211) as sturdy, luxuriant, rust-resistant, non-stoloniferous, highly fertile perennials, offering a cereal-grass of immense value, at least for fodder. The flowers are open-pollinated, but close-pollination does not reduce fertility. This series of amphidiploids has been grouped under the name *Agrotriticum*, the wheat species of the cross adding the species name, *e.g.*, *Agrotriticum durum* (211). Dodecaploid-amphidiploid *T. vulgare* ($2n = 42$) × *A. intermedium* ($2n = 42$) has also been obtained (402, 403, 407, 430).

Amphidiploids have been used to advantage in pollinating sterile undoubled hybrids, depending for success on the chance fertilization of an unreduced ♀ gamete of the primary F_1 hybrid by a reduced ♂ gamete of the amphidiploid (198, 206, 349, 386). The number of strains of amphidiploids may also be increased in this manner.

Back-crossing in amphidiploids (193, 195, 284, 349, 378) depended in part on parental races and direction of the cross for success and fertility.

Phenotypic expression of amphidiploidy in cereal crosses has been briefly referred to under the respective crosses. An almost infallible reaction to chromosome doubling in the primary cross is some degree of increased fertility (454) which in some series, it seems, is more apparent in the lower chromosome groups. The 42- and 56-chromosome wheat amphidiploids had generally a higher fertility than the 70-chromosome (594). However, other causes besides high polyploidy apparently lower fertility. Sears (454), in comparing the characteristics of 18 amphidiploids of diploid interspecific and intergeneric hybrids in Triticinae, observed that fertility varied from nearly perfect to almost zero. Also, there was no consistent relationship between the fertility of the amphidiploid and the lack of pairing in the undoubled hybrid.

Size and vigor of plant, thickness and greenness of leaf, maturity time, disease immunity, size and composition of grain, baking quality of meal, and general responses to external surroundings were apparently conditioned by the two components of the cross.

The grain size of fertile amphidiploids frequently was larger than that of the parents (247, 598). Sears (453) found that 12 of 17 tetraploid amphidiploids, interspecific and intergeneric, had grains exceeding in size those of either parent. The 1,000-grain weight of amphidiploid *Triticum orientale* ($2n = 28$) × *T. Timopheevi* ($2n = 28$) was 80 to 95 grams, with selected grains attaining 100 to 110 grams (591, 594), and exceeding the grain weight of either parent. Sears (453) found that intergeneric amphidiploids showed generally less increase over parental averages than interspecific. The 1,000-grain weight of amphidiploid *T. vulgare* ($2n = 42$) × *Agropyron intermedium* ($2n = 42$) was 10 to 20 grams, and intermediate between the 32 to 37 grams of *T. vulgare* and 5 to 6 grams of *A. intermedium* (407).

Flowering date is usually postponed with increasing degree of polyploidy. Kostoff (247) observed that diploids bloom later than haploids, tetraploids later than diploids, and amphidiploids later than the primary undoubled hybrid. Amphidiploids of winter varieties of *Triticum vulgare* × *T. Timopheevi* were late ripening spring wheats (594). Wheat amphidiploids generally had a longer vegetative period than their parents, and some combinations acquired greater capacity for winter survival (594). That polyploid plants are generally possessed of a greater degree of hardness has not been confirmed by Bowden (54). Chromosome doubling may secondarily affect the hardiness of a plant, but it is thought that genic mutation and hybridization bring about most of the variation in degree of resistance to cold. Löve and Löve (295), however, through a statistical study came to the conclusion that the percentage of polyploids in the flora increased with latitude. The perennial and biennial habits of plants in a genus, according to Müntzing (343) in a study of 582 species, including 48 genera, are more often associated with a higher chromosome number. The average gametic chromosome number of perennial plants was 16.95, of biennial and sub-perennial 15.5, and of annual 10.65.

Randolph (428) has suggested that increased chromosome duplication in already highly polyploid plants may be detrimental rather than beneficial. Most cultivated forms of wheat and oats are natural hexaploids, and further doubling leads to dwarfness and various deformities as well as high sterility. Kostoff (255, 256) is of the opinion that high polyploidy, and especially autopolyploidy, in plants with long chromosomes, as in the small-grain cereals, tends to lead to sterility, due in part to higher chiasma frequency and hence more multivalents and irregular chromosome distribution at meiosis. Highly polyploid plants with short chromosomes, therefore, should have a better chance of survival. Numerous evidences were drawn from natural polyploids with short and with long chromosomes. It may be pertinent in this connection that Marshak and Bradley (308) found the total chromosome length in the nuclei of natural wheat species decreased per genom with the increase in polyploidy. The mean aggregate length of the metaphase chromosomes of a root-tip nucleus was 118 μ in *T. monococcum* ($2n = 14$), 203 μ in *T. dicoccum* ($2n = 28$), and 248 μ in *T. vulgare* ($2n = 42$).

Stomatal size and distribution, generally fair indicators for autopolyploidy in cereals, seemed not too reliable as a test for amphidiploidy. Amphidiploid sectors of *T. monococcum* x *Ae. uniaristata* indicated no deviation from the undoubled hybrid as to stomatal size and distribution. Amphidiploid *Aegilopoides* x *Ae. umbellulata* showed generally fewer stomata per unit area, while amphidiploid *Ae. caudata* x *Ae. umbellulata* and *Ae. speltoides* x *Ae. umbellulata* had definitely larger and sparser stomata (449, 453). Stomatal size of decaploid *Triticum-Agropyron* amphidiploids exceeded that of the undoubled hybrids (406). The amphidiploid *Secale montanum* x *T. durum* had larger stomata than either of its parents (599). The mean stomatal length in *Triticum* was found to have increased with the natural increase in polyploidy from 41.7 μ in diploids, to 46.1 μ in tetraploids, and 53.3 μ in hexaploids (51). The stomatal number per unit area correlated inversely at 82.1 per sq. mm. for diploids, 63.9 for tetraploids and 46.6 for hexaploids.

Pollen grain volume, another indicator of cell size, presented in 56-chromosome *Aegilotricum* and its parents, *Triticum dicoccoides* and *Aegilops ovata,* a ratio of 2.47, 1.30 and 1.00, respectively (193). The pollen grain size apparently correlates with natural increase in polyploidy in the genus *Triticum,* for although variations occurred within a chromosome group the length and width in diploids averaged 47.7 x 41.2 microns, in tetraploids 54.4 x 47.6, and in hexaploids 61.7 x 54.3 (509).

The chromosome stability of amphidiploid offspring, in general, is variable with race combinations of the undoubled cross. Sears (454), in a tabulation of 21 different interspecific and intergeneric amphidiploids in the tetraploid group, found that out of 144 offspring 29 were monosomics, five were trisomics and the balance were of regular chromosome constitution. The preponderance of monosomics was attributed to loss of chromosomes during meiosis.

Sesquidiploids or Haplo-Diploids

In sesquidiploids the chromosome complement of one component of the cross has attained diploidy and the other has remained haploid. Sesquidiploids have been assumed to have arisen in several ways but usually through the fertilization of an unreduced gamete by a reduced gamete. The unreduced ♀ gamete may have been that of a natural species, as in case of the 49-chromosome sesquidiploid *T. dicoccum* (14 + 14)♀ × *T. vulgare* ($n = 21$) ♂ (168), or the unreduced ♀ gamete of a hybrid which on backcrossing to one of the parents has given rise to the sesquidiploid (50, 208, 209, 211, 281, 507). The parentage of the doubled complement can be inferred when the chromosome numbers of the two parents differ, and while it has generally been the ♀ gamete that has remained unreduced, cases have been reported in which the ♂ gamete may have remained unreduced, as indicated in the 35-chromosome sesquidiploid *T. vulgare* ($n = 21$) ♀ × *T. monococcum* (7 + 7) ♂ (234), or two sperms may have functioned, as suggested for the origin of the 49-chromosome sesquidiploid twin seedling *T. vulgare* ($n = 21$) × *T. armeniacum* (14 + 14) (190).

Triple Hybrids and Bridge Crosses

Some species do not cross directly with one another, but their chromosomes can be brought together through an intermediary bridge cross. Thus *Secale cereale* × *Haynaldia villosa* ended in failure, but [*Triticum dicoccum* (genoms AB) × *Haynaldia villosa* (genom V)] F_1 × *Secale cereale* (genom S) ♂ resulted in a robust trigeneric hybrid possessing characters of the three component genera (238, 267, 268). The 28 chromosomes, representing the complete genom sets of the three genera, remained as univalents in about 90% of the sporocytes, indicating little or no homology among the genoms ABVS. The success in bringing together the complete genoms in a triple hybrid of this type depends on the occasional formation on an unreduced ♀ gamete, probably through a restitution nucleus in the F_1 of the primary or bridge cross. Selfing of spontaneously unreduced gametes of the trihybrid, or subjection of plant to a polyploidogenic agency should bring into being a fertile trigeneric octoploid amphidiploid with the genom formula AABBVVSS.

A sterile perennial F_4 wheat-*Agropyron* plant with a somatic chromosome number of 48 pollinated with pollen of *Elymus giganteus* ($2n = 28$) gave rise to a trigeneric hybrid with 52 chromosomes. It was inferred that an unreduced ♂ gamete may have participated in the fertilization (508*a*).

It is desirable to transfer the high disease resistance of the Russian wheat *T. Timopheevi* to hexaploid wheats, but the two wheats do not cross successfully. However, [*T. spelta* (ABC) × *T. polonicum* (AB)] F_1 × *T. Timopheevi* (AG, or A*B*) ♂ was highly fertile with 28–34 and possibly 35 chromosomes (212). The highest number, 35, should represent the A and B genoms from paired, and C genom from unpaired chromosomes of the primary F_1, in addition to AG (or A*B*) genoms of *T. Timopheevi*. Through the pentaploid bridge cross the genoms of *T. vulgare* and *T. Timopheevi* were brought together. Similarly sterility in *T. monococcum* ($n = 7$) × *T. vulgare* ($n = 21$) was avoided by the previous introduction of a 28-chromosome wheat into a bridge cross with *T. monococcum* (234, 237).

The comparatively frequent formation of unreduced gametes in the F_1 of some of the *Triticum-Agropyron* crosses made these crosses on pollination with a third species amenable to the inclusion in a triple hybrid of the complete haploid genom sets of three species (208, 209, 211). As the unreduced gamete of the primary undoubled hybrid and the reduced gamete of the amphidiploid are identical, the triple hybrid type resulted also on crossing an amphidiploid hybrid with a third species, as briefly discussed under *Triticum-Aegilops* amphidiploids (328, 458, 507, 524). A number of other triple hybrids have been described by Vakar (554).

CHROMOSOME MORPHOLOGY AND GENOM RELATIONSHIPS

The present methods of attack on cereal chromosome morphology are laborious and possibly not always proving directly as fruitful as hoped for in unraveling problems of phylogeny. The difference in chromosome size is not outstanding, and divergence in arm length occurs to the same degree in too many chromosomes. Satellited constrictions with or without nucleoli, and secondary constrictions in general, should perhaps be some of the most tangible means for the identification of cereal chromosomes; but many of these constrictions often either elude detection or may possibly be simulated by artifacts. The reports are at least conditioned by cytological procedures and possibly by race and ecological conditions. Levan (285) found that in *Secale cereale* one pair of satellites was observed readily, a second pair with greater difficulty, and that by exposing growing root tips to 0° C. for one to three days, additional satellite-like bodies appeared at the ends of the chromosomes. The latter bodies brought out by cold treatment were possibly heterochromatic and their visibility due to nucleic acid starvation induced by low temperature treatment.

Triticum monococcum has been credited variously with none to four or more pairs of satellited chromosomes (67, 104, 388, 389, 495), and observations as to secondary constrictions in general have been far short of unanimous. The average of obtainable reports indicates that the number of total secondary constrictions may be approximately the same in diploid, tetraploid (49, 67, 74, 104, 160, 182, 184, 205, 223, 285, 388, 389, 464, 466, 495) and hexaploid (49, 223, 388, 464) chromosome groups, including species of *Triticum Hordeum, Secale, Aegilops* and *Avena.* Tandem satellites have been observed in a pair of chromosomes in *T. dicoccum* and *T. vulgare* (49). The maximum number of nucleoli was observed to be one per genom of seven chromosomes in diploid *Secale cereale* (388) ; tetraploids, *T. dicoccum* (49), *T. durum* (182, 184, 388), *Aegilops cylindrica* and *Ae. ovata* (388) ; and hexaploids, *T. vulgare* (49), *T. spelta* (388), *Ae. crassa* (388) and *Agropyron repens* (463). The diploids, *T. monococcum, T. aegilopoides* and *Ae. speltoides,* were exceptions in that they had two nucleoli per genom. Pathak (388) interpreted the presence of a maximum of two nucleoli per genom in some diploids as additional support of the theory that five is the basic number, and that seven is a secondary derivation in the Gramineae. Bhatia (49) found two large and two small nucleoli in *T. dicoccum,* four large and two small in *T. vulgare,* and suggested that the difference in size as well as staining reaction may be explained by the difference in origin of their respective genoms.

Kakhidze (182) investigated the possibility of chromomere arrangement in the pachytene of microsporocytes of *T. durum* as a method of comparing chromosome homology. Two pairs of SAT-chromosomes, distinguished from one another by the nucleolar contact body, were selected for comparison. Small sections on either side of the contact body showed differences in the two pairs as to sequence arrangement of large and small chromomeres, but whether these differences point to non-homology or to regrouping due to inversions can be determined only after the entire chromosomes have been studied.

Bodies designated as compound chromomeres have been differentiated in early and late metaphase of mitotic chromosomes of rye root tips subjected to 0° C. for 24 hours, fixed in strong platinoformalin or other suitable reagents and critically differentiated in the staining procedures (467, 468). The cold treatment also brought out secondary constrictions in addition to compound chromomeres (466). The compound chromomere was regarded as an aggregate of ultimate chromomeres. Shmargon (467) calculated that in the nucleolus-bearing SAT-chromosome of rye, approximately 50 ultimate chromomeres of the meiotic pachytene in the sporocyte were condensed into 11 compound chromomeres or packets of the early or late mitotic metaphase of the root tip. In split portions of the thread the size and distribution of compound chromomeres were identical (468). The shortest chromosome of the rye genom contained nine compound chromomeres, and the longest 13. The number of chromomeres between chromosome constrictions was constant. Comparisons of compound-chromomere arrangement in an appendaged chromosome of two varieties of *T. durum* revealed that a structural deficiency had occurred as to one of the chromosome arms of one variety (184).

Kostoff (246, 248, 249), through critical stain differentiation, observed deeply staining bodies which were grouped more closely at the ends of mitotic chromosomes in wheat. He interpreted these bodies as heterochromatin, and as possibly inert with the genes located between them.

Genom formulae generally evolve from studies of meiotic pairing in hybrids and comparisons of chromosome morphology in species. They may at best be considered as useful though perhaps only temporary markers of progress in classification and interpretation of added data. The use of different races under diverse conditions naturally precludes unanimous results and interpretations, thus rendering the problems more elusive, but perhaps also more challenging.

Idiograms indicate that 14 chromosomes, presumably genoms A and B, of *T. vulgare* resemble closely the chromosomes of *T. dicoccum* (49) and *T. polonicum* (287). Chromosome morphology as well as apparent autosyndesis in hybrids seem to indicate that three chromosomes in the A genom may closely resemble three of the B genom (287), but the chromosomes of the C (or D = dinkel) genom are all unlike those of the A and B. The B genom of two tetraploid wheats *T. Timopheevi* and *T. armeniacum* has a limited homology with the B genom of other tetraploid wheats and has been designated as genom *B* (238–241, 243) or genom G (220, 425, 516). Kihara (220) has suggested that hexaploid wheats derived the AB genoms from *T. dicoccum* and the C genom (or D = dinkel) from an extinct or yet undiscovered form in Afghanistan or Iran. As existing AABB plants are rust-resistant, lack of resistance in AABBCC plants must be due to the C genom. CC plants may be extinct as a result of low resistance, but if extant must possess some rust-resistant character which could be utilized in breeding for rust immunity. Tetraploid *Aegilops cylindrica* has the C genom, and Johnson (175) found this species highly resistant to some races of rust. Pathak (388), after a study of chromosome morphology, proposed the idea that *T. vulgare* is a hybrid between the diploid *Ae. squarrosa* and a tetraploid form of *Triticum,* the latter having previously arisen as a *Triticum-Aegilops* hybrid. *Ae. squarrosa* has hollow stem and low rust resistance, and it is further suggested that it may also be one of the parents of the tetraploids *Ae. cylindrica* and *Ae. crassa* with which it occurs in Turkistan. It is needless to comment that the origin of hexaploid wheats is still an unsolved enigma and anybody's guess.

That close interrelationships exist among certain species of *Agropyron, Triticum* and *Aegilops* is evident (116, 564). The apparent autosyndesis of X_1 with X_2 *A. elongatum* chromosomes in the F_1 meiosis of *T. vulgare* × *A. elongatum* has led Vakar (548) to suggest that *A. elongatum* is an amphidiploid hybrid, $A_aA_aB_aB_aC_aC_aX_1X_1X_2X_2$, between a tetraploid with the genom formula $B_aB_aX_2X_2$ and a hexaploid with the genom formula $A_aA_aC_aC_aX_1X_1$ such as *A. intermedium.* Peto (400), though apparently studying other races of the *Agropyron* species, also came to the conclusion that *A. elongatum* is an amphidiploid that possibly arose through hybridization of an *intermedium-* (syn. *glaucum*) like plant, AAXXYY, with a 28-chromosome *Agropyron,* XXYY, and the subsequent doubling of the F_1 genoms AXXYY to produce the 70-chromosome amphidiploid.

Haynaldia villosa and species of *Secale* stand apart in that their genoms V and S, respectively, have no counterpart in intergeneric crosses thus far studied (235, 238–242, 267).

Idiograms of *Avena* suggested a close relationship between diploid and tetraploid species (464). *A. barbata* appeared to have two very similar genoms, differing only as to one chromosome, and is probably autotetraploid and derived from a diploid which may also have been a progenitor of *A. strigosa.* The hexaploid oats *A. sativa* and *A. fatua* seemed to have only 18 pairs of chromosomes in common with respect to morphological likeness, but the morphological dissimilarity of the remaining three did not prevent pairing. The hexaploid oats thus far studied differ from the diploid and tetraploid in possessing a larger number of heterobrachial chromosomes.

Haga (154) points out in regard to the stability and mutability of the genom that certain differentiations as a whole of the subgenoms of an allopolyploid lead eventually to a new composite genom functionally diploid and no longer divisible into ancestral genoms though still polyploid as to chromosome number.

More recent discussions concerning the origin, interrelationships or geographical distribution of cereals are available as to 28-chromosome wheats (48), including *T. Timopheevi* (114); the 42-chromosome wheats (105, 106, 140, 141, 293, 446); *Agropyron* (387, 564); *Secale* (275, 446); *Hordeum* (395, 446); *Aegilops* (218, 220, 223); and *Avena* (76, 111, 341).

ANEUPLOID AND STRUCTURAL CHROMOSOME ABERRATIONS

Aneuploidy frequently accompanies directly changes in euploidy, auto- or allo-, but is due also to other factors that disturb the chromosome balance, whether these factors are of natural origin and apparently remote, or induced and immediate. The aneuploidy may be temporary or more or less permanent.

Secale cereale

Some races of *Secale cereale* have a strong tendency to shift from the basic chromosome number seven to eight. Sometimes these 16-chromosome races present further aberrations, as fragmentation and asynapsis (118). Müntzing (357), in a study of 167 rye individuals, found that 69 showed some form of meiotic irregularity. Two plants were trisomic for a SAT-chromosome, and one plant had an additional pair of chromosomes. In a dwarf 15-chromosome plant the trisomic state supposed to have arisen through non-disjunction also apparently involved the SAT-chromosome (518). At diakinesis usually half of ♂ sporocytes had six bivalents and one trivalent, and the other half had seven bivalents and one univalent. A plant with two fragments of chromosomes (355) gave rise to plants without any fragments or up to eight of them. The increase in number of fragments had a depressive effect on the plant, expressed chiefly by lowered fertility. The fragments were apparently subinert. A plant with one fragment (356) gave rise to plants with new types of fragments which were believed to have arisen through misdivision of the centromere of the fragment, thus forming telocentric chromosomes which in turn through misdivision of the centromere developed into iso-chromosomes. More recently Müntzing (356*a*), while studying intravarietal crosses involving plants with standard chromosome fragments, has concluded that there may be an old and widely distributed standard type of extra chromosome in rye, and that the deviating types of fragments sometimes observed are more or less ephemeral deviations from the standard. A striking property of the standard fragment in material studied was its ability to increase numerically by non-disjunction at some post-meiotic stage. Experiments strongly indicated that the process of non-disjunction is directed in such manner as to generally pass the fragments to the ♂ and ♀ gametes. The tendency of the directed post-meiotic non-disjunction to increase the number of fragments in the offspring is counteracted by the meiotic tendency to eliminate the fragments. In the Östgöta Gråråg variety the negative effect of meiosis coupled with lowered fertility of plants with fragments seemed to be greater than the positive effect of post-meiotic non-disjunction. In the Vasa II variety, on the other hand, good pairing at meiosis, due probably to genotypical control, seemed to favor fragment increase in the offspring. In Östgöta Grågåg the large iso-fragment derived from the long arm of the standard fragment exhibited post-meiotic non-disjunction, but the small iso-fragment derived from the short arm of the standard did not.

Inbreeding in rye appeared generally to favor various forms of chromosome aberrations (183, 204, 276, 426) exemplified by low chiasma frequency and presence of univalents, chromosome bridges and altered chromosomes. Kakhidze (183) observed that inbred individuals of "Vjatka" rye varied widely as to number of chiasmata and other meiotic characteristics. Homology of chromosomes of diploid genoms should increase with inbreeding. However, as the accumulation of genes in a homozygous state may cause depression or stimulation exhibited phenotypically in changed fertility, *etc.*, the genic constitution should likewise be reflected in meiosis. The existence of genes causing variations in number of chiasmata and consequently irregular chromosome distribution would through inbreeding isolate individuals differing from one another in the process of meiosis. The general depressive effects of inbreeding would also probably be a contributing factor in rye.

Camara (70) observed that subjection of plants to high temperature of 35°–45° C. greatly increased the inherent instability of rye, and as heat of this magnitude arises in nature it may be responsible for the production of 16-chromosome rye. Popoff (422) attributed extra chromosomes in Bulgarian rye populations to spontaneous crossing with *Secale montanum.* Kostoff (245, 269) found trisomics in the progeny of *Secale cereale* x *S. montanum,* and also one plant with nine normal-sized chromosomes in addition to the expected 14. As *S. montanum* does not grow wild in Sweden, Müntzing (355) concluded that Popoff's theory probably does not apply to aneuploids found in the Swedish rye populations, and is more inclined to believe that fragments are one of several symptoms of cytological unbalance characteristic of *S. cereale,* and possibly other allogamous species. Races with 16 chromosomes disappear, but are frequently reproduced anew by fragmentation and other structural and numerical chromosome aberrations.

Speltoids, Compactoids and Fatuoids

Huskins and Smith (164) attribute the normal head type of *Triticum vulgare* to a balance between ear-lengthening and speltoid glume factors, of unknown chromosome location, and the ear-compacting and round glume factors borne on the long arm of the C-chromosome. In some cases the unbalance may be the result of the mutation of a gene (544). In other cases it is ascribed to numerical or other quantitative deficiencies or duplications in the head-type-determining chromosome in one or more of the three genoms. Uchikawa (545–547) has proposed formulae for various types of speltoids and compactoids studied by him. The off-type heads were frequently accompanied by aberrant chromosome numbers (65, 66, 201, 322, 545–547, 559), but sometimes by deficiency or duplication in a portion of one or more of the chromosomes concerned (69, 300, 544). Sears (457), in his intensive study of nullisomics, identified the speltoid-suppressing chromosomes as one of the 14 homologous with the 14 in the 1*n* complement of *T. durum* and designated it as IX in his series. Nullisomic IX was hence a homozygous speltoid. The chromosome, the loss of which results in speltoidy, apparently in this case belonged to genoms A or B. Matsumura (322), in a study of pairing in hybrids, has reached a similar conclusion in regard to another nullisomic speltoid. Love (300) found 42-chromosome speltoids with a slightly deficient heteromorphic pair in the progeny of a varietal cross. He believes that deficiencies of this type are likely to occur in hybrids due to breakage at the attachment region in unpaired chromosomes, and to fragmentation due to cross-over in inverted segments. Some of the 42-chromosome speltoids (A series) may have deficiencies too small to be detected cytologically. Camara (69), in the progeny of a pentaploid cross, also found speltoidy associated with a heteromorphic pair. He believes that translocation is the primary cause of speltoidy and that the loss or addition of chromosomes is secondary; the more frequent occurrence of speltoids in northern countries with low temperature may support this theory. Ellerton (105) suggests that the frequent formation of a quadrivalent in the F_1 of *T. sphaerococcum* × *T. vulgare* may be the result of a reciprocal translocation which involved a chromosome associated with the speltoid character, and that this translocation is responsible for a proportion of heterozygous speltoid mutants in F_2. Also, as all the *sphaerococcum* characters in the above cross behaved like one recessive gene, Ellerton proposed the theory that *T. sphaerococcum* itself arose as the results of a chromosomal deletion in the population of *T. vulgare* forms which spread into northwestern India from Afghanistan.

Speltoids in *T. vulgare* have their counterpart in fatuoids characterized by wild-oat type of "grain" in cultivated *Avena sativa.* Sander (440) has explained the rise of these aberrants in *A. sativa* as follows: "Alterations in the normal balance between the C chromosome and the rest of the complex give rise to fatuoid, steriloid, and sub-fatuoid mutations. These changes are produced by deficiencies of various degrees in the C chromosome and behave genetically as multiple allelomorphs". Thus removal of the wild-type inhibitors through loss of all or part of the long arm of the C-chromosome (163) permitted the expression of steriloid, fatuoid or sub-fatuoid characters in varieties of *A. sativa* and *A. byzantina.* Nishiyama (374), in a study of four *Avena* "grain" types, cultivated, intermediate, wild and naked, found that the loss of a pair of C-chromosomes, whether lost from the cultivated, intermediate or wild types, resulted in each case in the wild type. He suggested that in the cultivated *Avena* type the C-chromosome is associated with the cultivated type of "grain" characters, and a B-chromosome is associated with the wild type. In the wild type both the B- and C-chromosome carry the wild type of "grain" factors. In the intermediate type the C-chromosome carries a gene group for intermediate instead of cultivated factors. Experiments indicated that the gene or gene group for naked grain is also associated with the B-chromosome.

Monosomics, Nullisomics and Polysomics

Monosomics ($2n-1$) in which one chromosome of a pair is missing, and nullisomics ($2n-2$) in which both members of a pair are missing, rarely, if ever, exist as diploids. In a polyploid such deficiencies may have depressive effects expressed by reduced vigor or fertility, *etc.*

Sears (450) obtained monosomics by pollinating a haploid of the hexaploid *T. vulgare* with "diploid" pollen, and nullisomics by selfing the aberrants. Thus 17 of the 21 possible nullisomics have been produced (457). Eleven of the found nullisomics (I–IX) involve the A and B genoms, and six (XV–XX) involve the C genom. All are reduced in vigor, but none is completely sterile. Nullisomic III caused asynapsis and thus provided an additional source of monosomics. Several genetic factors of *T. vulgare* have been located as to their respective chromosomes (455, 457) through this study.

Some monosomics are characterized by various degrees of aberrancy in head (65, 322, 545, 546) and "grain" types (371, 374). In the monosomic offspring of *Avena sativa gigantica* × *A. fatua* (408) the structurally changed and missing chromosome dominantly controlled leaf width, with the result that the nullisomics had narrow leaves.

Polysomics, in which one or more chromosomes appear in triplicate or higher multiple, may, in contrast to numerical deficiency, occur also in diploids. Trisomic plants in the diploid barley showed at meiosis misdivision of chromosomes of the trisome, and other irregularities (500). In a 15-chromosome sterility group of barley the gametes with the extra chromosome were usually sterile (205).

Sears (450, 457) obtained trisomics directly in the progeny of a haploid *Triticum vulgare* ($1n = 21$), and from a nullisomic (nulli-III) pollinated with the diploid. Tetrasomics arose in the progeny of trisomics with about the same frequency that nullisomics occurred in progeny of monosomics, namely 1% to 10%. It seemed, however, that chromosome duplications were transmitted through fewer ♀ gametes and more ♂ gametes than were deficiencies. Tetrasomics frequently formed quadrivalents, but otherwise meiosis was fairly regular. In the progeny of six tetrasomic plants of different origin, 36 of 44 were tetrasomic. Most of the trisomic and tetrasomic plants were more like the 42-chromosome plants than were the plants with deficiencies. Tetrasomics sometimes compensated in part for nullisomics. Polysomics, like deficiencies, may involve the chromosomes that govern head types (66, 201, 545).

Other Structural Chromosome Aberrations

Observations on chromosome morphology and meiotic chromosome behavior, especially in numerical aberrants, leave little doubt as to the magnitude of the part played by structural aberrations in chromosomes. Camara and Coutinho (74), in a study of eight tetraploid wheats, found considerable variation in chromosome idiograms from species to species. The similarity or dissimilarity did not always coincide with genetical results. They concluded that tetraploid wheats may differentiate subspecies by chromosomal rearrangement, brought about through inversions which would originate deletions, duplications and translocations; by point mutations; or by crossing-over between partially homologous regions. According to Love (300), most and possibly all hybrids between *vulgare* wheats are heterozygous for one or more inversions. In fact there were indications that all varieties of tetraploid and hexaploid wheats used (302) differed to a greater or lesser degree in arrangement of chromosome segments, thus presenting favorable conditions for deletions and other structural chromosome changes. Müntzing (357) found in rye populations plants heterozygous for small inversions as well as for segmental interchanges.

Autopolyploidy accounts for trivalents and higher multiples in some cases, but many others are apparently the result of structural changes in one or more of the chromosomes involved (88, 302, 404, 450, 451, 489, 496, 499, 500, 525, 527). Crossing-over in more or less dissimilar chromosomes (197, 228, 244, 302, 370, 372, 408, 451) in auto- or allosyndesis no doubt figure in these structural chromosome changes. Kostoff (246) suggested that the comparatively dense heterochromatin at the ends of chromatids may be responsible for end to end pairing in haploids and in certain cases may lead to chiasma formation. The association of heterochromatic portions of non-homologous chromosomes in hybrids might lead to interchanges and production of forms with reorganized karyotypes. In haploid *Secale cereale* heterochromatin ends apparently played a part in pachytene pairing of non-homologous chromosomes (285). Frequency of sporocytes with multiple chromosome associations in different pentaploid wheat hybrids varied from less than 3% in Hope × Vernal, to more than 90% in Marquis × Pentad (302). The suggestion was offered that the multiple associations arose through pairing of chromosomes which were phylogenetically similar but not strictly homologous. Telokinetic chromosomes (189, 302, 304, 356, 457) resulting from loss of one arm through misdivision of the centromere (189) may give rise through a second misdivision to isochromosomes with two identical arms. Sears (457) obtained a telocentric or an isochromosome or both from 15 of the 17 identified chromosomes of *T. vulgare,* most frequently in the offspring of some monosomic individuals. Univalent chromosomes resulting from hybridization in wheat were found to be a fruitful source of telokinetic chromosomes (302, 304). As a result of breaks at the kinetochore one or both arms might bear a spindle attachment and participate in the subsequent nuclear divisions. Terminal kinetochores were, however, usually unstable.

Altered chromosomes of more unusual types sometimes occur (204, 426). A chromosome in which a peculiarly shaped end appeared to function in some ways as a centromere was discovered in rye (426).

Fragmentation, a common phenomenon associated with other structural chromosome disturbances, has been observed in amplified forms in certain mutations (498).

ASYNAPSIS, DESYNAPSIS OR DISSOCIATION

Asynapsis is characteristic of meiosis in many F_1 hybrids and progenies where low homology and structural differences interfere with chromosome pairing. There are other cases where conditions are seemingly amenable to bivalent formation yet univalents are abundant at first metaphase. The chromosomes may be paired at middle prophase but fall apart before the entry of the chromosomes on the spindle. The terms desynapsis and dissociation may better describe these latter types of asynapsis.

A strain of rye showed pachytene pairing, but by metaphase many of the pairs had dissociated in both ♂ and ♀ sporocytes (425). The dissociation was controlled by a single recessive factor. An apparently spontaneous mutation in F_3 of a varietal cross in a hexaploid wheat was characterized by a high state of asynapsis at first metaphase with the number of pairs, mostly of the end to end type, varying from zero to 21 (289). Normal prophase pairing occurred in all the plants, but in some of them desynapsis began at pachytene and in others at diplotene. The aberration was a simple Mendelian recessive character. There were, possibly, modifying genes present. Fertility was lowered in varying degrees. Other dissociation mutants appeared in the progeny of interspecific hybridization and as result of X-ray treatments in diploid wheats (495–498). A nullisomic of *T. vulgare* was partially asynaptic (nulli-III) (457). Factors affecting synapsis in oats were located in the C-chromosome (163), which also in part controls "grain" types.

The delicate balance in both unstable and stable pairing may be disturbed by external conditions. Li, Pao and Li (289) found the gene governing desynapsis most effective at low temperatures approaching 10° C., and only then was desynapsis complete. Prakken (425) ascribed an increased frequency of univalents in an asynaptic rye during one season to high temperature and low water content in soil and air. Fixations on different dates showed that environment influenced significantly the meiotic instability in varieties of common wheat (360). Inbreeding in rye favored lower chiasma frequency and increase in number of univalents (276). Bondarenko (53), in surveying the fertility of *Triticum-Agropyron* hybrids, concluded that all life processes in the organism and especially chromosome conjugation are dependent upon environmental conditions.

STERILITY

Gamete and seed sterility may at least in part be attributed to chromosome aberration in some form. Hybrid sterility in general has been ably reviewed by Thompson (522).

Defective pollen tube growth may be an immediate cause of pollen sterility, and is apparently a contributory cause of self-sterility in rye (448). Matsumura (323, 327) observed that pollen of *Triticum spelta* ($2n = 42$) germinated far better than pollen of *T. polonicum* ($2n = 28$) on the stigmas of *T. polonicum, T. spelta* and the F_1 hybrids of the two. The pollen of the hybrid germinated very poorly.

Histological studies have revealed that failure to obtain hybrids between apparently incompatible species may not be due to failure of fertilization. In *Triticum-Elymus* hybridization experiments fertilization took place, but the embryo and endosperm either failed at the beginning or developed through the earlier stages and then aborted (413). Failure in *Hordeum* x *Secale* was due to early death of embryos following abnormal development of the endosperm and its degeneration after five or six days (61–63, 91, 523). The most striking feature of the endosperm development was the failure of nuclear division to keep pace with chromosome division, thus increasing the polyploidy of each of the few nuclei formed to 100 or more chromosomes and one to 20 nucleoli (526). Among suggestions offered as to causes of endosperm and embryo degeneration (526) are that some kind of immunity reaction may take place between ♀ plant and hybrid tissue, or that ♀ plant may not produce the right kind or right amount of food for the hybrid with a different genetic constitution. Pissarev and Vinogradova (411) were able to obtain *Triticum-Elymus* hybrids beyond the embryonic stage only when each parent plant had developed from an embryo transplanted onto endosperm of the respective foreign genus. The spring wheat Lutescens 62 in hybridization with rye gave 25% success when grown from embryos grafted upon rye endosperm, but only 4.3% when grown from ungrafted embryos.

Success in obtaining hybrids between plants of unlike chromosome numbers frequently depends on the direction of the cross. The autotetraploid *Secale cereale* ($4n = 28$) ♀ x *S. cereale* ($2n = 14$) ♂ set seed, but the reciprocal was unsuccessful due to failure of pollen tube growth (85). In *Hordeum* ♀ x *Secale* ♂ the embryo developed to a certain stage, then failed to develop further (526). In the reciprocal even inception of endosperm and embryo failed. Auto-"triploid" *Triticum vulgare* (42+21) ♀ × *T. vulgare* ($2n$ = 42) ♂ produced a grain set of 68.8%, but the reciprocal was sterile (569).

The grain set may be good but the germination a failure. Pentaploid grains were produced more freely when the tetraploid wheat was the ♀ parent, but grain viability was poor. The reciprocal with the hexaploid wheat as the ♀ parent produced fewer but viable grains (50, 55, 462, 522). Similarly the direction of the cross conditioned the hybridizing results obtained in a triploid *Avena* hybrid (368); in *T. vulgare* ($2n$ = 42) × *Secale cereale* ($2n$ = 14) (55, 522); also in back-crossing of pentaploid wheat hybrids to their tetraploid and hexaploid parents (319), and octoploid *Aegilotricum* to its tetraploid components (193).

The immediate cause of embryo inviability, in whatever stage of development, of low-chromosome ♀ × high-chromosome ♂, it seems, is the abnormal development of the endosperm (55, 368). The primary causes apparently lie with unfavorable relationships as to chromosome numbers in embryo, endosperm and maternal tissue. In contrast to the above results, *Triticum-Agropyron* hybrids were obtained with greater ease when self-pollinating *Triticum* served as the ♀ parent, irrespective of chromosome numbers (34, 211, 279). Only *A. elongatum* among the *Agropyron* species crossed with *Triticum* was found to be self-pollinating, and then only partially so (494).

Crossability in general is often greatly influenced by varieties (493). In crosses with *T. Timopheevi* grain set varied from zero in one strain of *T. spelta* to 93% in one strain of *T. compactum* (521). *Triticale* (Rimpau) ♀ × *Secale cereale* ♂ gave no grain set, but its substitution by *Triticale* (Meister) ♀ gave 23% (284). Fortunate choice of parental strains augmented grain set from 10% or less to 90% or more in crosses between *Triticum* and *Agropyron* (561). Race was found to affect success of the tetraploid *Triticum-Secale* crosses (98, 558). Rye pollen gave germination of 10% and 60%, respectively, on stigmas of two varieties of common wheat, Marquis and Chinese (55).

For some of the cases of sterility definite genetic bases have been discovered. When crossed with Petkus rye Marquis wheat ♀ produced less than 3% grain set, while Chinese wheat 466 ♀ produced 60% or more. Marquis ♂ gave no grain set, but Chinese 466 ♂ gave 2.2% grain set with 50% germination. Lein (283) attributed the crossability of Chinese 466 to two genes designated as kr_1 and kr_2. Male sterility in the form of shrunken rudimentary anthers, in a *Hordeum* mutant, was found to be a recessive factor segregating three normal to one ♂ sterile (514, 515). Bagged ♂ sterile plants were completely sterile, open-pollinated highly fertile. Another sterile form of *Hordeum* (205) bore a gene, carried by the pollen, that disturbed the embryo sac development, possibly by interfering with the antipodal tissue formation. Varieties of *Triticum monococcum* were observed to possess genes which were dominantly lethal when introduced into a cross with *Aegilops umbellulata,* causing the death of the hybrid embryo (452, 456). *T. aegilopoides,* a species closely related to *T. monococcum,* did not carry these genes, and neither did some of the segregates from *T. monococcum* x *T. aegilopoides.*

The self sterility of *Secale* may in part be attributed to weak pollen-tube growth caused by an inhibitory substance produced by the ovary (448). This substance exerted the strongest influence in the lower style where germination was frequently prevented. However, as pollen tubes sometimes entered the embryo sac but without subsequent grain development (278), other factors also were apparently responsible for the sterility and were further aggravated by self-fertilization. Müntzing (348) found that pollen sterility in varying degrees was common in rye populations and was inherited. About half of 610 plants examined could be classed as partially ♂ sterile. Ovular sterility correlated weakly with pollen sterility. Landes (278) conceded that weak pollen-tube growth in selfed *Secale* was in part the cause of sterility, but found also that 5%–10% of ovaries examined had no embryo sac. Twice as many embryo sacs aborted in the selfed as in the cross-pollinated florets.

Aneuploidy may in some instances cause sterility. In trisomic *Hordeum* eight-chromosome gametes were usually sterile (205). Poor or shrivelled grains of autotetraploid *Secale* were more frequently associated with aneuploid embryos than were plump grains (354). Hypotetraploid embryos favored shrivelling more than hypertetraploid. Similar correlations were observed in the diploid and aneuploid progeny of triploids, and may in general be due to disturbed balance of chromosome numbers in respect to embryo, endosperm and surrounding tissue.

Certain structural chromosome changes frequently influence fertility, due to mechanical disturbance of meiosis. Reciprocal translocations following X-rays and intercrossing of progeny in *Triticum monococcum* were found to lower fertility (497, 499). A ring of six chromosomes produced an average sterility of 31%, a ring of eight chromosomes 51%, a ring of ten chromosomes 66%, two rings of four chromosomes 24%, and one ring of six together with a ring of four chromosomes 48%. No ring resulted in only 5.5% sterility. In 4,200 cells 73% had rings arranged in a zigzag manner. This disjunctional division of chromosome complexes reduced the sterility materially below that expected for random segregation (499, 525).

Meiosis in *T. Timopheevi* ($2n = 28$) x *T. armeniacum* ($2n = 28$) was remarkably regular and tetrads were apparently regular, but most of the pollen was entirely deformed (516). It was thought that pollen sterility might have resulted in this case because the chromosomes of one species could not be substituted for those of another, due to divergences in the parental forms developed through gene mutations and structural chromosome changes.

External conditions may also temporarily influence fertility. *T. durum* x *T. Timopheevi,* sterile under ordinary field conditions, was under high soil fertility induced to produce some grains (579, 580). However, even under favorable cultural conditions the undoubled *Triticum Timopheevi* x *T. durum* remained highly sterile compared with the corresponding amphidiploid (588). Excellent nutrition apparently cannot overcome the cytogenetical causes of sterility. Use of pollen from later-generation offspring, and to a lesser extent the artificial breaking of the F_1 anthers, favored increased fertility in the above F_1 hybrid (580). X-rayed pistils of *T. spelta* produced approximately 20% grain set when pollinated with *T. Timopheevi,* whereas the control was negative (520). It was suggested that the X-rays had weakened the inhibitory action of the hexaploid stigma toward the diploid pollen. Sterility of varieties of wheat, grown under adverse conditions of northern Caucasus and regions within the arctic circle, was ascribed to failure of fertilization of the polar nuclei (28). These nuclei were much elongated and densely surrounded by cytoplasm.

POLYPLOIDOGENIC, ETC., AGENCIES

Chemicals

The most extensively used polyploidogenic chemical since its widespread introduction in 1937 (156) is colchicine derived chiefly from *Colchicum autumnale.* In U.S.S.R. alone, during a brief period, more than 90 new amphidiploid wheat types have been produced by means of colchicine treatment (594). The effect of colchicine on the cell dynamics has been described by Beams and King (42), Derman (96) and Beal (41), and its chemical nature by Shmuck (469). Some aspects of the molecular structure of colchicine distinguish it sharply from other alkaloids, and at the same time approximate it to another group of substances, the sex hormones, and the carcinogenic hydrocarbons (469). Some of the histological features induced by colchicine present an analogy to those found in cancerous animal tissues and some plant galls and tumors (156, 252, 469).

In cereals colchicine has been applied usually in concentrations of 0.05–0.5% in aqueous solutions to dry, or to water-soaked and germinating grains (42, 57, 78, 119, 145, 188, 252, 325, 342, 409, 449, 461, 584, 586), usually for 24 to 48 hours. It has also been injected into coleoptiles (64, 392) and applied to crowns of plants (453) or florets (325). In a mixture with lanolin it has been employed in salve form (453). Pulp of *Colchicum* employed as a germinating medium produced doubling of chromosomes of wheat and rye (259).

The colchicine treatments have resulted in highly varying degrees of success. The balance between polyploidogenic and lethal effects is delicate. Outstanding success was achieved by Greis (145) who obtained 14 tetraploids and 18 chimeras from 100 treated barley seeds. The best concentration was 0.4% for 48 hours on grains in early stages of germination. Excellent results were also attained by Sears (453) by treating eight to 11 mm. long coleoptiles of diploid F_1 hybrids with 2% by weight of colchicine powder in anhydrous lanolin for 24 hours before planting in soil. Another highly successful procedure was to apply to crowns of potted plants, before jointing, cotton saturated twice daily with 0.5% aqueous colchicine solution for four or five days. This method was advantageous in rare hybrids in that the plant could be divided so as to increase the number of individuals. The treated plants were usually chimeras with only some heads or parts of heads polyploid to furnish seed for the completely polyploid generation.

Apiol (132–134) and its isomers extracted from parsley, *Petroselinum crispum* (syn. *Apium petroselinum*), brought about anomalies similar to those produced by *Colchicum*. Parsley contained sufficient polyploidogenic substances to produce tumors, and increased polyploidy in wheat grains germinating in its vicinity (132).

Convallarin, a glucoside derived from *Convallaria*, in 0.5% and 0.8% aqueous solutions produced on root tips of wheat seedlings small tumors of multinucleate cells but no polyploid nuclei (5).

Of the artificially synthesized chemicals acenaphthene has been tested most widely (136, 207, 247, 250–253, 365, 469, 470, 486). The general morphological hypertrophies and cytological aberrations were similar to those produced by colchicine. The solubility in water is only about 0.003%, and hence the crystals may be scattered over the moist plant organs without danger of too high dosage. Acenaphthene is less expensive and less toxic than colchicine and has shown indications of being more selective in its effect as to plant species. Growth was inhibited by its application in wheat, but not in peas, beans or lentils (172). *Colchicum autumnale*, unaffected by its own polyploidogenic substance, colchicine, responds to acenaphthene with disturbed mitosis and increased polyploidy (207).

Many other chemicals have been tested as to polyploidogenic properties. Shmuck and Gusseva (471) have synthesized more than 100 different carbocyclic and heterocyclic substances. The chemicals are presented with structural formulae and tabulated to show their respective biological effect on wheat, and their analogy to the respective carcinogenic substances. Some of the compounds affected alike all plants tested; others affected one group and not another. The cereals, wheat and barley, were highly responsive; the legumes, peas, vetch, and clover, least so. The chemical nature and biological effect of synthetic substances are further discussed by the above (472–477) and other authors (117, 130, 131, 135, 137, 138, 282, 482, 483, 485–487).

One small group of chemicals, certain mercuric compounds, deserve mention, as they are active ingredients of some fungicides. "Ceresan" contains ethyl mercury phosphate and caused hypertrophy in oat and other seedlings when used in aqueous suspension of 1:2000 (444). "Granosan" contains 2% of ethyl mercury chloride and when used in aqueous concentrations of 0.5–0.1% (equal to 0.01–0.002% ethyl mercury chloride) for three to six days caused the usual polyploidogenic results in cereals (260, 264, 447). "Niuifa", another mercuric seed disinfectant, produced similar results (447).

X-rays

X-ray irradiation as a polyploidogenic agency is apparently more effective at lower dosages of 250 to 500 r (2, 3). With 1,000 r and upward the frequency of polyploid cells in rye decreased (59, 60). Among 533 rye seedlings X-rayed at 250 r for the express purpose of obtaining polyploids, two developed into tetraploid plants and other individuals had polyploid sectors (56). In root tips of *T. monococcum* also haploid cells and sectors were observed (67).

In cereals X-rays have usually been applied to air-dried grains or to seedlings. At low dosages of 250 to 500 r, the number of mitotic cells in rye increased greatly with a subsequent increase in yield (58–60). Following dosages of 1,000 r or more, chromatin abnormalities increased, and rate of mitosis and final grain yield decreased. Exposure of air-dried grains of *Triticum durum* to 16,000 r reduced the frequency of cell division 42% (6), and markedly retarded growth. Exposure of wheat spikes to 16,000 r produced nuclear disturbances in about one third of the florets (8). The resulting pollen grains were of diverse sizes and chromosome numbers, and the grain yield was reduced. Sterility of ovaries in barley was greater when X-rays were applied to heads immediately before flowering than when applied at earlier phases of plant development (410).

The X-ray effect was persistent, as X-rayed dormant grains of *T. vulgare* stored several months to three years before being allowed to germinate showed no decrease in the cytological irregularities induced by irradiation (4, 7).

Certain factors have been found to influence the effect of X-ray irradiation. Covered grains of barley and oats, after dosages of 10,000, 15,000 and 20,000 r, gave a markedly higher germination percentage than hulless grains (128). Larger grains with correspondingly larger embryos in two varieties of wheat survived 20,000 r with less mortality than smaller grains. Higher water content of grains, affecting their metabolic activity, increased mortality at higher dosages (127, 139, 150). Associated with the lethality were meiotic disturbances. At 10,000 r grains of barley containing 10% of water showed 12.66% of mitotic disturbances in germinating embryos; 15% of water, 27.99%; and grains soaked 23 hours, 53.8% (139). The percentage of nuclear aberrations in barley increased also with each added year of storage of grain before X-ray irradiation (151, 152).

As referred to in the discussion of autopolyploids (350, 353, 503), tolerance to X-rays in general increases with degree of polyploidy. Marshak and Bradley (308) noted that the degree of inhibition of mitosis in wheat seedlings was inversely proportional to chromosome number. It was neither inversely nor directly proportional to the aggregate chromosome length of the chromosome complement, which in the hexaploid nucleus was almost twice that of the diploid nucleus instead of three times as should be expected on the basis of genom number. There was evidence to indicate that the inhibitory action of X-rays on mitosis might take place through the centromeres. The importance of the centromere in the initiation of mitosis is indicated by the behavior of micronuclei during mitosis. Micronuclei retain structural integrity until the major nucleus of the cell enters prophase. Simultaneously with the major nucleus the micronuclei go through the characteristic changes if their chromosomal components contain centromeres, if not they become pycnotic and disintegrate. The theory was proposed that radiation will reduce the number of mitosis-initiating centers, which may possibly be identified with centromeres, in proportion to the number present in the cell. Thus if one half of the centromeres are inactivated, seven should still be intact in the diploid, 14 in the tetraploid, *etc.*, and inactivation of a sufficient number of centers to cause mitotic inhibition would require a heavier dosage in the tetraploid.

X-ray-induced chromosome abnormalities, unlike X-ray-induced mitotic inhibition, increased with the degree of polyploidy. The number of chromatic bridges (503) in root-tip mitosis of wheat seedlings following exposure to 10,000 r increased with each rise in genom number from an average of 0.7 per cell in diploids to 1.7 in tetraploids and 2.2 in hexaploids. Likewise the frequency of translocation rings resulting from fertilization of wheat florets with X-rayed pollen rose with each succeedingly higher degree of polyploidy. Fröier, Gelin, Gustafsson and Tedin (126, 129) found that at 5,000 r the number of disturbed nuclei in seedling roots of X-rayed wheat grains was directly proportional to the degree of polyploidy. At 15,000 r, however, the amount of nuclear disturbance had risen more rapidly in the diploid than in the hexaploid. The seeming discrepancy might be explained by the discovery by Marshak and Bradley (308) that the total chromosome length per genom was considerably less in the hexaploid than in the diploid, and also by the general observation in various plants and animals that the frequency of X-ray-induced chromosome abnormalities varies directly as the total length of the chromonemata of the somatic chromosome complement.

The germinating and sprouting ability of the hexaploids remained unaffected even when more than every second mitosis was disturbed (126). In the diploid *T. monococcum* there was a pronounced decrease in growth, even with few nuclear disturbances and low X-ray dosages.

A Mendelian factor in *T. monococcum* increased the susceptibility of dormant seeds to X-ray injury (501), but seemed to have no effect on mutation rate.

Among the various forms of X-ray-induced nuclear disturbances some involved specifically chromosome aberrations, numerical as well as structural, including fragmentation, inversions, bridges, translocations, *etc.* (67, 139, 151, 503).

Camara (71, 72), in a study of two pairs of SAT-chromosomes in *T. monococcum,* concluded that the two regions of most frequent breaks were one near the centromere and the other near the distal end. The region near the centromere also had a higher power of fusion. SAT-1 chromosome was less resistant to X-rays than SAT-2 chromosome.

X-ray irradiation has been a profitable method of obtaining mutations. Some of the mutations conspicuously affected meiosis in form of extensive chromosome fragmentation, partial or complete asynapsis at metaphase, diploid spores and chromosomal rings (441, 495, 498). Other mutations included abortion of anthers before or after meiosis (498); shortening of growth period, thus allowing growth of more than one generation per year (410, 496, 498); change from spring to winter habit (306); high resistance to *Erysiphe* (123); chlorophyll deficiencies (150, 152, 153, 178, 353, 410, 495, 498); dwarfs (178, 410); fatuoids (95); tubular heads (410; speltoids, *etc.* (441, 495, 498). Factors as biotypes (441), water content of grain (152) and age of grain were sometimes effective in determining the type of mutations obtained.

Temperature

Natural climatic temperatures have been credited with altering the chromosome status in some instances. Garnet wheat grown in Schleswig-Holstein showed 24% of sporocytes with univalents, but only 5.8% when grown in its native Canada (44). Mention has been made of extreme natural temperatures in connection with spontaneous rise of speltoids (69) and haploids (429). Root tips of *Hordeum bulbosum* fixed during cold weather showed a low chiasma frequency and a large number of univalents (45).

Experiments on the effect of temperature on the induction of chromosome aberrations have been limited mainly to heat, including ranges from 35° to 50° C., and in one case of dormant grains a temperature of 80° C. (503). Some treatments involved sudden extreme temperature changes (402, 403). Exposure time varied from 20 to 60 minutes, to several hours or to several days. Heat was applied most frequently to the spikes at some chosen stage between premeiosis and early post-pollination. Grains were treated in dormant stage (503) or allowed to germinate at the high temperatures (399, 401).

Root tips of rye seedlings germinating at 35° to 36° C. showed fractured chromatids, translocation, abnormal chiasma formation, *etc.* (399). Treated heads of rye frequently gave rise to abnormal meiosis including structural chromosome changes (77) and decrease in number of chiasmata to complete asynapsis (70). Kagawa (179) reports diad formation in two heat-treated plants as averaging 3.5% and 8.6%, respectively, as against 0.3 to 1.6% in four controls. Sparsely among the progeny of heat-treated plants there arose "tetraploids" (99, 121, 185, 186, 358, 402, 403), numerical chimeras (68, 181, 186, 401) or haploids (375). Heat alternated with cold resulted in one amphidiploid (402, 403). Tolerance to heat, contrary to the response to X-rays, was not favored by higher chromosome numbers (503).

High temperature that produced a number of mutants in two strains of *T. compactum* produced none in a variety of *T. vulgare*

(181), indicating perhaps differences in reaction between the two species. Low temperature greatly increased the effectiveness of a gene governing desynapsis in a wheat mutant (289). The effect of low temperature in differentiating compound chromomeres and amplifying secondary chromosome constrictions has been briefly referred to under chromosome morphology.

Centrifugal Force

Ultracentrifugal force of 150,000 to 400,000 times gravity in stratifying the cell contents displaced the spindle to the end of the cell (42). The metaphase stage frequently recovered the normal state. The late anaphase stages often suffered permanent disruption as the two nuclear masses were displaced more strongly centrifugally away from the spindle which through displacement or inactivation failed to initiate cell wall formation. The result was often binucleate cells, and sometimes multipolar spindles in the subsequent division.

Centrifuging caused structural chromosome changes in *T. monococcum* (73). Chromosomal breaks occurred more extensively during the first 12 hours following the treatment. Fusion of breaks did not follow immediately. Wheat grains, at point of breaking seed coats, centrifuged for various periods at 3,000 revolutions per minute, lost in survival value with increasing doses (66). Various mutants, including aneuploid speltoids, were obtained.

Miscellaneous Agencies

Germinating seeds of common wheat placed in a tube with both ends attached to electrodes and subjected to electric currents of varying intensity gave rise to some aneuploid speltoids and other mutant plants (65). The rate of mutation tended to increase with exposure, more evidently so when direct current was applied.

Grains of wheat exposed to the light of the spark of an induction coil resulted in mutants characterized in part by higher resistance to lodging and diseases, and by 10 days earlier maturity (115).

Seedling roots of barley immersed in 1 : 75,000 aqueous solution of neutral red for one hour, then grown in dark, showed mitotic abnormalities as pycnosis, anaphasic bridges and pseudomitosis, not seen in proper controls (391). Cell division ceased after seven hours.

Barley seedlings grown in an oxygen-free atmosphere of nitrogen showed various nuclear abnormalities, as clumping of chromosomes, sticky anaphase bridges and extrusion of chromatic material (511, 512). Seedlings treated four days recovered after a few days in normal air, but a six-day exposure was lethal.

The cytological aberrations associated with extended seed storage have been well summarized by Crocker (94). Aberrations appeared in rye sometimes after only two to three years of storage, and included structural chromosome changes (366). In one lot of eight-year old seed, 43.8% of mutants developed.

EMBRYO SAC, EMBRYO AND ENDOSPERM

During the past decade more cytomorphological studies have been directed toward the development of the embryo and related structures.

The rate of pollen-tube growth is apparently variable with plant race and growth conditions. In rye pollen-tube growth was generally completed within 24 hours after pollination (448). In barley pollen germination began five minutes after reaching the stigma, and in 45 minutes one ♂ gamete was in contact with the egg, the other with the two fused polar nuclei (415). The most rapid pollen-tube growth in barley took place at 30° and 35° C. when the ♂ nuclei were present in embryo sac 20 minutes following pollination (420). At 5° C. the same growth required 140 minutes. The triploid endosperm nucleus showed division figures within six hours and the zygote within 14 hours (415).

The development of embryo sac and early post-fertilization stages have been illustrated in wheat (28, 338), rye (43), normal (331, 397) and sterile (205) plants of barley, and embryo-endosperm relationships in reciprocal crosses involving parents of unlike chromosome numbers (55). In countable stages nuclear division occurred three times as often in the endosperm as in the embryo, and Pope (420) suggested that the extra set of genes in the endosperm may be the major factor in its rapid growth. The optimum temperature for growth seemed to be about 30° C. One hour's exposure to 40° C. brought about signs of injury to egg and polar nuclei.

Antipodal tissue, highly developed in Gramineae, probably functions directly in the early nutrition of the endosperm, and indirectly in the nutrition of the embryo (62, 91, 526).

Plump grains without embryos are found in small numbers in cereals. Some varieties produced very few to none of these sterile grains, others produced up to 3% in some years (148), and the rate of occurrence was also higher in cross-pollinated plants like rye. The immediate cause of embryoless grains is assumed to be single fertilization whereby only the endosperm develops. Hybridity and growth conditions are modifying factors. Single fertilization may also affect the egg, in which case no endosperm forms. Endospermless grains, however, are lost in screening due to their light weight and shrivelled state. Experiments on the inheritance of spontaneous embryolessness in spring wheats did not give conclusive results.

Apomixis, reported in some members of the Gramineae, was observed among perennial segregates of the back-cross (*Secale cereale* × *S. montanum*) × *S. cereale* (263). Small leafy plants with rootlets occupied the place of anthers and ovaries in the spikelets, and developed into normal individuals when planted. The apomixis and vivipary in these segregates were induced by low temperatures of 0°–10° C. At temperatures above 15° C. normal sexual reproduction took place. Induced vivipary of normal but immature embryos of barley varieties possessing extreme dormancy was brought about by exposing the embryo end of the ovary to constantly wet filter paper (417, 421).

The feasibility of obtaining viable grain from cut flowering culms stored in water has been demonstrated by Pope (414, 418). The satisfactory storage of ovaries and pollen on cut culms at low temperatures for 21 days or longer may be of distinct advantage in hybridization of forms with unlike ripening dates (416).

It now seems possible that rescue may be in store for some of the many cereal hybrid infants finding conflicting forces too turbulent for a happy existence. Embryos of *Hordeum jubatum* ($2n = 28$) ♀ × *Secale cereale* ($2n = 14$) ♂ normally died before maturity (61, 63). One of several embryos excised from caryopses and grown in cultural solution, however, developed into a mature plant. Barley embryos of different ages, grown on artificial media, produced small seedling-like plants (331). Cell differentiation was more distinct in the cultured embryos and aided in the interpretation of the development of naturally grown embryos.

Observations were made on the state of chromosomes of oat embryos during dormancy (508) and initial germination (513).

Summary of work on endosperm of Gramineae, to year 1939, has been presented by Alexandrov (11). Alexandrov and Alexandrova have made extensive studies on the endosperm, especially in *Triticum,* including earlier stages of formation (21, 23, 25) and later stages of development (339). They have sought to determine the histological structure and the status of ergastic substances, as starch and protein in relation to flinty and floury endosperms (14, 15), endosperms of soft and hard wheats (22) ; of full and meager grains (16, 17), and of grains produced by plants with short and long ripening periods (24). As the endosperm matured, the nuclei, especially in the deeper layers, first increased in size, then became deformed, shrank, died and finally disintegrated (19). Formation of small starch grains continued during the nuclear disintegration.

The endosperm of some species of *Agropyron,* as *A. intermedium* and *A. elongatum,* seemed very similar to that of *Triticum.* Both large starch grains of plastid origin and very small grains of chondriosomal origin were present (18). In other species, however, as *A. cristatum,* the chondriosomal starch was absent or very scant, and a calcium oxalate druse apparently replaced the nucleus at maturity.

One characteristic of the high-baking qualities, in contrast to low-baking qualities, of some Russian wheats was the lower amount of chondriosomal starch and higher degree of protein intercalation in the endosperm of the high-baking grain (169). Jakolev (170) observed that in several species of wheat cross-pollination favored the development of less fine-grained starch as well as of a larger proportion of protein. Heads were selfed on one side and emasculated on the other to favor cross-pollination with other individuals of the same variety.

Rudiments of protein grains were detected in oat endosperm 14 or 15 days after pollination (396). One grain appeared in the center of each of numerous, probably proteinic, vacuoles in the cytoplasm of the aleurone cells.

Amylolytic activity and sugar compositions were determined in wheat species of the three chromosome groups (231). The amylolytic activity was lowest in the diploid *T. monococcum.* Maltose contents as well as total sugars were high in tetraploid *T. durum* and *T. polonicum;* low in diploid *T. monococcum;* intermediate in tetraploid T. *dicoccum* and *persicum,* and in hexaploid T. *vulgare, sphaerococcum,* and *spelta.*

Transplanting of embryos to foreign endosperm has been found to reduce grain dormancy (573). Germination was advanced more by transplanting to non-dormant than to dormant grain types. Various morphological and physiological responses have been induced in endosperms in caryopses of plants grown from embryos grafted onto endosperms of other genera (411, 478). The endosperms of grains born by plants of graft origin acquired some of the characteristics of the adopted endosperm, as to pigmentation of grain, quality of gluten, quantitative relationship of starch and protein, *etc.* It has been suggested that the phytohormone action of the borrowed endosperm might be the main cause of these changes. The transplants were made between dry caryopses, the foreign embryo being cemented into the position of the replaced native embryo with paste made from flour of the same genus as the adopted endosperm (411).

The advantage of large endosperm in spring wheat was demonstrated by increased vigor and yield attained when an extra endosperm was joined to the one already possessed by the embryo (146).

Xenia in small grain cereals (52, 81, 124, 309) is apparently not readily discernible. The phenol color tests, used to distinguish grains of different wheat and barley varieties (233), when applied to grains of wheat crosses produced the same color reaction as grains of the maternal plant (336). The grain from the F_1 plants was colored irrespective of the direction of the cross.

Cyto-histological studies dealing more exclusively with pericarpic structures have been made available by Alexandrov and Alexandrova (10, 12, 13, 19, 20, 26, 27). Varietal differences in the structure of the wheat grain are illustrated by Bates (40).

Flowers with two or three carpels developing into fertile grains have been found in several lines in the progeny of a varietal cross in T. *vulgare* (332). The percentage of flowers with multiple caryopses averaged about 70.5 in earlier generations.

VEGETATIVE GRAFTS

Aside from the grafting of entire embryos onto endosperm, embryonic, as well as hypernodal grafting on young culms, also has been described (165, 412).

MITOTIC PERIODICITY

A study of mitotic periodicity, in root tips of month-old seedlings, indicated primary diurnal periods of high mitotic activity shortly after 12:30 A.M. and 12:30 P.M. in *Hordeum vulgare, Triticum vulgare,* and *T. spelta,* and apparently none in *T. monococcum* and *Secale cereale* (437).

SUMMARY

Added study of cereal cytogenetics has further substantiated the apparent fact that with the great powers of stability inherent in the chromosome architecture there are allied equally great forces for numerical and structural changes, implying inevitably genic redistribution. Many of these alterations escape detection under present cytological methods.

Deviating chromosome numbers of spontaneous origin are conspicuously demonstrated in natural species. One source of euploid changes is polyembryony. While on the average some 3,000 cereal grains may be required to produce one set of multiple embryos and nearly 60,000 grains to produce one seedling with an altered chromosome number, these seemingly scant aberrants may become an impressive factor if now and then through the centuries a survival chance occurs. Also, some races produce a much higher number of caryopses with multiple embryos. Outside the phenomenon of polyembryony, too, natural causes bring about euploid aberrancy, operating either through the reproductive mechanism or through vegetative mutation expressed in form of chimeras.

Artificial means of producing chromosome changes have been applied with increasing endeavor. Among polyploidogenic chemicals colchicine has so far produced the most satisfactory results in raising the degree of euploidy. Acenapthene has been found to be more effective in cereals than in some other plant groups. Reports are available also as to the efficacy of numerous other chemicals, including some well known disinfectants.

Increase in euploidy has sometimes followed extreme temperatures and extreme temperature changes.

While X-rays in higher dosages are very effective in bringing about general mutations, it is subsequent to low dosages that euploid chromosome changes have directly been most frequently observed, either as tissue sectors or entire plants.

Many cereal haploids, spontaneous or induced, have been observed. Though these highly unstable and often less robust replicas of the female parent have no immediate agronomic value, some recent experiments indicate that they may become highly important in the cytogenetic analyses of the species or race, as well as outstanding in demonstrating the instability of chromosomes. "Triploids" also should probably be classed in the category of valuable experimental material.

Some cereal "tetraploids" may become agriculturally important. Morphological and physiological comparisons indicate that some striking differences occur between the "tetraploid" and its parental diploid. The forms and directions that these differences may assume seem to be conditioned by the species and race involved, and probably also by the ecological environment of the individual plants examined.

There are indications that very high euploidy in plants with long chromosomes, as in "small grain" cereals, is unfavorable to plant vigor and survival value.

Hybridization apparently is one immediate cause of numerical chromosome changes. Natural hybrids are reported in cereals and their near relatives. In artificial production of hybrids the main emphasis at present is placed on conversion of sterile and unstable F_1 hybrids into more or less fertile and stable amphidiploids. The number of these auto-alloploids is rising so rapidly that a babel of new generic and specific terms is foreseen, unless plant breeders and taxonomists collaborate universally in the naming of the new mongrels and also in the preservation of a few replicas in well regulated herbarial galleries.

The various amphidiploids may be objects of great hope or of equally great doubt as to valuable agronomic prospects. Some show immediate promise; others may have hidden values to be discovered only in the distant future. Historically, even those natural forms man has found ready made and most to his liking he has seen fit to recondition even more to suit his desires. On contemplating time and monetary value spent on collecting and culturing plants which ten years ago were labeled as discards or useless, one wonders on passing a discredited wayside weed how soon it too will emerge as a highly respected member of the chosen or its genes will circulate with those of the supposedly best.

Triple hybrids and bridge crosses have been found useful in bringing together the chromosomes of two highly incompatible species, the third species serving as the intermediary. One such triple cereal hybrid includes the chromosome sets of three genera. One purpose of such crosses is to introduce into a favorable agronomic race some desirable character, as hardiness, drought resistance or disease immunity.

Aneuploidy also occurs in natural species. Among cereals some races of common rye almost habitually attain an extra chromosome or one or more fragments of chromosomes. The aneuploidy may disappear only to reappear in later generations. Several theories have been proposed as to the basic cause of this easily detected form of chromosome instability apparently inherent in races of rye.

Aneuploidy frequently follows in the wake of other chromosome aberrations, numerical or structural. The loss or gain of a chromosome or two may express itself phenotypically, as in some speltoids, compactoids and fatuoids.

No less profound than numerical changes and morphologically evident structural changes as to eventual results are the internal chromosome reconstructions, large and small. Some of these internal alterations are detected with more or less difficulty by observing the form of the meiotic pairing. The interpretations to be placed on the pairing procedures demand considerable cytological and genetical familiarity with the material studied before fairly accurate conclusions may be offered. Many modifying influences may affect pairing, some of which may be external, as temperature, others internal, as genic control.

Advisably more attention is being extended to the reproductive morphology of cereals and other grasses. These studies should prove fruitful in discovering more of the basic causes of various types of sterility, including failures of hybrid embryos, and the effects of the intricate nuclear interactions among embryo, endosperm and ovular tissues in hybrids between parents of like as well as unlike chromosome numbers.

Little progress may seem to have been made during the past ten years in the unravelling of cereal phylogeny. Yet the reporting of discoveries of new natural species and the synthesis of 42-chromosome wheats similar to the vulgare and spelt types are definitely forward steps.

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[1] Also invaluable in the preparation of this review have been the Bibliography of Agriculture, the U. S. Dept. of Agr. Library, Biological Abstracts and Plant Breeding Abstracts by presenting translations of titles in brackets and by giving reviews of subject matter unavailable to the reviewer on account of language barriers or other causes; and the Bibliofilm Service of the U. S. Dept. Agr. Library.

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THE BOTANICAL REVIEW

VOL. XII JUNE, 1946 No. 6

SPECIALIZATION, HYBRIDIZATION, AND MUTATION IN THE CEREAL RUSTS*

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INTRODUCTION

The small-grain cereals, wheat, oats, barley and rye, are among man's most important food-producing plants. As the several rusts to which they are subject are the most serious diseases of these plants and perhaps the greatest hazards in their production, it is but natural that they should have received more scientific attention than the rusts of any other group of plants. Intensive study of these rusts during the last half century has yielded a great and steadily growing fund of knowledge concerning them. It is the purpose of this paper to review, as briefly as is consistent with clarity, the main contributions to our knowledge that have accrued from two important discoveries—that of physiologic specialization, and the discovery of the existence of heterothallism in the rusts. The former showed these rusts to be made up of numerous parasitically different strains; the latter opened the way to studies on hybridization between these strains, which in turn have thrown considerable light on their genetical constitution.

POLYMORPHISM AND HETEROECISM

Although rusts have affected cereals from prehistoric times to the present, scientific study of these important pathogens goes back only about 200 years. Hooke pictured the teliospores of a rust in his "Micrographia", published in 1665; and Micheli made a beginning at rust nomenclature when, in his "Nova Plantarum Genera", published in 1729, he gave the name *Puccinia* to a rust that later was transferred to the genus *Gymnosporangium* (4). Classification of the rusts, however, did not really begin until about the end of the eighteenth century when Persoon (164, 165) recognized the rusts as a distinct group of fungi and divided those known to him into several genera.

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During the first half of the nineteenth century much work was done on the taxonomy of the rusts, despite the fact that investigators, under the influence of the theory of spontaneous generation of lower organisms, generally did not accept rusts as living and self-propagating species of plants. Most of this work was later to undergo modification because the creators of new genera and species did not understand the life cycles of the rusts and consequently classified different spore forms that belonged to the life cycle of the same rust as different genera. The realization that a single rust might possess more than one spore form was brought about chiefly by the epochal work of the Tulasne brothers and Anton de Bary. The Tulasnes accepted the common association of urediospores and teliospores as evidence of their common origin (222, 223) and described the production of the sporidia by the promycelia of germinating teliospores (221). They showed, in other words, that some rusts, at least, were polymorphic. The fact that some species of rust spend a part of their life cycle on one host species and part on another was demonstrated by de Bary (10) who infected barberry with sporidia from teliospores of *Puccinia graminis* with the result that aecia of the species known as *Aecidium Berberidis* were produced. This experiment showed that the two previously accepted species of rust merely represented different parts of the life cycle of one rust. He thereby established the fact of "heteroecism", a name that he coined to describe the alternation of hosts in the life cycles of certain rusts. This discovery was followed by numerous demonstrations of rust species that produce pycnia and aecia on one species of host plant and uredia and telia on another (108).

SPECIALIZATION OF CEREAL RUSTS

Historical review

Species of rusts, as of other plants, are based on morphological characteristics. The fact that there exist within some morphological species well defined strains that can be differentiated from one another by physiological criteria (*i.e.*, the specialized ability to attack only certain hosts) was demonstrated in 1894 by Eriksson and Henning (46) and Eriksson (41) for *Puccinia graminis* Pers.

They showed that in Sweden there were several of these specialized strains and designated them as *formae speciales* (*forma specialis*, sing.). About the same time Hitchcock and Carleton (87) found in infection experiments conducted in Kansas that "both wheat and oats are easily infected by rust from the same kind of grain, but not by the same kind of rust from other grains", and they concluded that "the rusts of various cereals are probably physiological species". In the present paper these *formae speciales* or physiological species will be referred to as "varieties" of the rust.

In *P. graminis* specialization is not necessarily identical, though it appears to be rather similar, in different parts of the world. Eriksson (41, 42, 44) and Eriksson and Henning (47), as a result of experiments with European rust collections, divided the rust into the following varieties: *a*) *Secalis* (on rye, barley and certain grasses); *b*) *Avenae* (on oats and certain grasses); *c*) *Tritici* (on wheat); *d*) *Airae* (on *Aira caespitosa*); *e*) *Agrostidis*[1] (on *Agrostis* spp.); *f*) *Poae* (on *Poa* spp.); to which Eriksson (45) added a seventh variety, *Epigaei*, on *Calamagrostis* spp. In North America (189, 193, 204) the varieties *Tritici*, *Secalis*, *Avenae*, *Agrostidis* and *Poae* have been found as well as the variety *Phlei-pratensis* which also occurs in Europe but was regarded by Eriksson as a separate species In the United States there has recently been collected (49) on *Agropyron spicatum* what appears to be a new variety of *P. graminis*, differing pathogenically from those mentioned above. In Japan *Puccinia culmicola* Diet., with its aecia (*Aecidium Berberidis-Thunbergii* P. Henn.[2]) on *Berberis Thunbergii*, has been regarded as a variety of *Puccinia graminis*, owing to its ability to attack *Agropyron* and rye (6).

The differences between these varieties of *Puccinia graminis* are not solely physiological, for it has been demonstrated (47, 114, 191, 193, 204, 235) that they differ somewhat in the size of their spores, particularly the urediospores. The morphological differences, however, are not great enough to enable each variety to be identified except perhaps by means of statistical studies of the measurements of many spores.

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[1] Some writers quoted in this paper have used the ending *-is* for specific names based on *Agrostis* and *Calamagrostis* rather than that of *-idis* originally used by Eriksson (41). For the sake of uniformity the latter ending is used exclusively in this paper.

[2] Moreover, the Sydows (Monographia Uredinearum Vol. 4: 245. 1923) give the aeciospore wall as 1 μ thick to 2–6 μ at the apex, a feature characteristic of the aeciospores of *Puccinia graminis* on *Berberis vulgaris*. They comment: "Diese Art steht dem *Aec. Berberidis* Gmel. äusserst nahe und ist wohl damit identisch, da morphologische Unterscheide kaum wahrnehmbar sind".

Puccinia triticina Erikss. (*P. rubigo-vera Tritici* (Erikss. & Henn.) Carl.) is one of a group of gramineous leaf rusts belonging to the old species *P. rubigo-vera* (DC.) Wint. which Eriksson and Henning (46) in 1894 divided into the two species *Puccinia glumarum* Erikss. & Henn. and *Puccinia dispersa* Erikss. & Henn. The latter species Eriksson (41, 42) resolved into four varieties: *a*) *Secalis* (on rye); *b*) *Tritici* (on wheat); *c*) *Bromi* (on *Bromus* spp.); and *d*) *Agropyri* (on *Agropyron repens*). He later (43) revised this nomenclature by raising the variety *Tritici* to the status of a species (*P. triticina* Erikss.) as he did also the variety *Secalis* to which he gave the name *P. dispersa* Erikss. These two species are morphologically similar, but *P. triticina* produces aecia on *Thalictrum* spp. (93, 123) and, in Siberia, on *Leptopyrum* (*Isopyrum*) *fumarioides* L. (20), whereas *P. dispersa* forms aecia on *Anchusa* spp. (11, 124) and is also reported to attack *Echium tuberculatum* (156). As they are but two among many rather similar rusts with uredia and telia on gramineous hosts and aecia on the Ranunculaceae or Boraginaceae, some taxonomists (5, 34, 125) prefer to regard them as merely varieties of a complex species, as, indeed, Eriksson did originally. To this group of grass rusts Arthur (5), following Mains (123), has given the name *Puccinia rubigo-vera* (DC.) Wint., and Cunningham (34), the name *Puccinia Elymi* Westnd. In the present paper, however, the names *P. triticina* and *P. dispersa* are retained for the two cereal rusts, as this has been the custom in most plant pathological literature.

Puccinia glumarum (Schm.) Erikss. & Henn. is the only one of the cereal rusts for which no alternate host is known (77, 211, 218). As stated above, this rust was separated from *P. rubigo-vera* by Eriksson and Henning. It was divided by Eriksson (41) into five varieties: *a*) *Tritici* (on wheat); *b*) *Secalis* (on rye); *c*) *Elymi* (on *Elymus arenarius*); *d*) *Agropyri* (on *Agropyron repens*); and *e*) *Hordei* (on barley). Later investigations (70, 79, 145, 207, 208, 210, 211, 219) have shown that specialization in this rust is less clear-cut and definite than Eriksson supposed; and the existence of his varieties has been questioned. Nevertheless it has been shown (48, 210, 211) that certain strains of the rust have a preference for wheat, others for barley or for rye; and that specific strains occur on *Agropyron repens* and *Hordeum murinum*.

The specialization of *Puccinia coronata* Cda. is among the most complex to be found in the grass rusts. De Bary (11) first showed the ability of this rust to produce aecia on *Rhamnus.* Klebahn (107, 108) divided the rust into two species: *P. coronata* Kleb., with aecia on *Rhamnus Frangula;* and *P. coronifera* Kleb., with aecia on *R. cathartica.* More recent work (19, 35, 136, 209, 219) has failed to substantiate this division; and *Puccinia coronata* Cda. is now generally regarded as a species made up of several varieties, of which some produce aecia on different species of *Rhamnus,* while others form aecia on *Lepargyraea* or *Elaeagnus.* Cunningham (34) recognizes the six varieties *Agrostidis, Avenae, Calamagrostidis, Glyceriae, Holci* and *Melicae;* and Arthur (5) admits the additional varieties *Agropyri, Alopecuri, Festucae, Lolii* and *Phalaridis.* Brown (18) reports the occurrence in England of yet another variety, *Arrhenatheri.* Fraser and Ledingham (51) found the following varieties to occur in the Prairie Provinces of Canada: *Avenae* (aecia on *R. cathartica,* uredia and telia on *A. sativa* and *A. fatua*) ; *Calamagrostidis* (aecia on *R. alnifolia,* uredia and telia on *Calamagrostis* spp. and *Scolochloa festucacea*) ; *Bromi* (aecia on *Lepargyraea canadensis,* uredia and telia on *Bromus ciliatus, B. latiglumis* and *B. Porteri*) ; *Elaeagni* (aecia on *Elaeagnus commutata,* uredia and telia on *Calamagrostis elongata*).

In *P. coronata* there is some evidence that the pathogenic characteristics of at least some of the varieties may be changed by passage through the alternate host. Dietz (35) states that urediospores of *P. coronata Avenae* cause only subnormal infection on *Holcus lanatus;* yet aeciospores produced by the *Avenae* variety of the rust on several species of *Rhamnus* were able to rust this grass heavily. Similarly aeciospores of the variety *Calamagrostidis* showed greater ability to rust oats than did urediospores of that variety; and conversely aeciospores of the *Avenae* variety rusted *Calamagrostis canadensis* which *Avenae* urediospores normally do not. These results led Brown (19) to study the effect of passage through the alternate host on the pathogenicity of the varieties *Calamogrostidis, Holci* and *Alopecuri.* Her experiments, however, indicated "that the varieties of *Puccinia coronata* are not altered appreciably by their passage through the aecidial host". She found, furthermore, that these varieties did not readily intercross with each other.

Puccinia anomala Rostr. (*Puccinia simplex* Erikss. & Henn.)

with aecia on *Ornithogalum* (124, 217) is considered by most authorities to be confined in its uredial and telial stages to cultivated barley. There are, however, reports of its ability to attack certain wild species of *Hordeum* (79, 234), and d'Oliveira (154) has collected on *H. murinum, H. maritima* and *H. pratense* strains that parasitized these grasses but were unable to attack cultivated barley. These strains he regards as highly specialized races or varieties of *P. anomala*. He, however, did not demonstrate their ability to infect *Ornithogalum,* and some authorities might be inclined to look upon them as strains of *Puccinia Hordei* Fckl. (213).

From the time of Eriksson's discovery of specialization in *Puccinia graminis* in 1894 until 1917, the *formae speciales* or varieties described by him were regarded as the ultimate units of specialization. Realization that a further subdivision of these units could be made resulted from the discovery by Stakman and Piemeisel (203) of a strain of wheat stem-rust to which they originally gave the name *Puccinia graminis Tritici-compacti.* This strain differed from other wheat stem-rust that they had studied previously in that it produced weak infections on the hard spring wheats and the hard winter wheats (113, 204). When still other strains were found (118, 135, 141, 198) that could be differentiated by the reaction of wheat varieties, it became evident that the variety *Tritici* was specialized into physiologically or pathogenically different strains that could be distinguished by the reaction to them of varieties within the genus *Triticum* in much the same way as Eriksson's *formae speciales* could be distinguished by the reactions of genera or species of the family Gramineae. The economic significance of these discoveries stimuated the search for new "biologic forms", as they at first were called, with the result that 37 of them were described within five years (192). The term "biologic form" was later superseded by that of "physiologic form", which term eventually gave place to "physiologic race", the designation used in the present paper.

The study of intra-varietal specialization was soon extended to various other cereal rusts, with the result that every one of them is now known to be composed of a number of physiologic races.

Identification of physiologic races

An understanding of what is meant by physiologic races requires discussion of some of the factors involved in their identification.

As indicated above, physiologic races differ from each other pathogenically, and, as a physiological basis must be postulated for such differences, the term "physiologic race" seems appropriate. Even if all the hosts of a rust were physiologically identical, it would still be possible to identify a few physiologic races because pathogenically different strains of a rust produce different symptoms. If the host is entirely congenial to the rust, large uredial sori (uredia) are produced with little if any sign of chlorosis. If the host is not entirely congenial, the sori are smaller and an area of sharp chlorosis usually surrounds the uredial pustules. On an uncongenial host the rust will produce small uredia surrounded by necrotic areas. The fact that there is much physiological variation in the host as well as in the rust, makes it possible to identify numerous physiologic races. The various symptoms produced by a rust are known as "infection types" and are represented by symbols; and the different host varieties that have been found most suitable for displaying the diversity of infection types are referred to as "differential hosts" or "differential varieties". Infection types for use in the identification of physiologic races of *P. graminis Tritici* were described by Stakman and Levine, and these have been adopted generally for the other cereal rusts with whatever modifications are necessary to suit the individual characteristics of a rust. A description of the infection types, as applicable to stem rust of wheat, is given below:

0—Host immune
  No uredia developed; hypersensitive flecks usually present, but sometimes there is absolutely no trace of mycelial invasion in the host tissues.
1—Host very resistant
  Uredia minute and isolated; surrounded by sharp, continuous, hypersensitive necrotic areas.
2—Host moderately resistant
  Uredia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green but slightly chlorotic islands.
3—Host moderately susceptible
  Uredia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.
4—Host very susceptible
  Uredia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.
X—Host reaction heterogeneous
  Uredia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on re-inoculation small uredia may produce large ones and *vice versa*. Infection ill defined.

The first problem facing the original investigator of specialization in a cereal rust was that of discovering the particular host varieties most suitable for distinguishing physiologic races—a problem that usually required the testing of hundreds of varieties of the host. The differential varieties thus discovered differed, of course, for different rusts, and in some cases more than one group of differential varieties have been devised for the same rust.

It was soon found that a good differential host must possess certain characteristics if it is to be satisfactory for the distinguishing of physiologic races. Perhaps two of the most important characteristics are homozygosity, and stability of reaction to rust under fluctuating environmental conditions. The first can be obtained rather readily (except in rye varieties) by selection; the second can be secured only by rigidly discarding varieties whose reaction responds too readily to environmental influences.

Physiologic specialization in Puccinia graminis Tritici

The discovery of the presence of physiologic races in the *Tritici* variety of stem rust has already been discussed. As this is one of the most widely distributed of the cereal rusts and second to none in economic importance, it is natural that its specialization should have been studied intensively. In the United States and in Canada, surveys to determine the prevalence of physiologic races have been conducted annually from 1919 to the present time; and in Australia data are available for the period 1922 to 1938. In several other regions—South Africa (229), British East Africa (Kenya) (111, 112, 128, 129), India (133), the Argentine (225, 226)—surveys have also been carried out for periods of years. As a result of these studies and others too numerous to mention here, at least 200 physiologic races of this rust have been identified. Not all of these were collected from uredia or aecia gathered in nature. A considerable number were produced in experimental hybridization studies, and more than 50 of the races so produced have not yet been found to occur naturally.

As far as it is possible to judge of the pathogenic characteristics of the races present in different regions from comparisons of their infection types on the 12 wheat varieties used as differential hosts, it would appear that there are rather pronounced pathogenic differences between those occurring in widely separated regions. The two emmer wheats, Vernal and Khapli, are resistant to all the physiologic races that have been common in North America since surveys of races were undertaken, the latter variety being resistant to all the races found in North America. In other regions, however, certain races attack these varieties severely. In South America (the Argentine, Brazil, Uruguay) race 42, which attacks Khapli heavily, at least in the seedling stage, is common (225, 226), and race 189, which attacks both Vernal and Khapli, has been found in Peru (56). In Abyssinia five races that attack Khapli have been described (188), two of them rather closely resembling race 42 which was originally discovered in Egypt as was also race 41 which likewise attacks Khapli (56). In India (133) race 42 is more common than any other race, and the two races next in order of prevalence attack the variety Vernal heavily. In Europe race 40, which attacks Vernal heavily and is extremely rare in North America, is one of the most commonly collected races (55, 81, 157). Examples of pathogenic differences in the rust need not, however, be confined to widely separated continents. It has been shown (205) that races which attack the variety Marquis heavily are very rare in southern Mexico but occur commonly in the northern part of the country—a fact indicating a very small amount of interchange of inoculum between the two regions.

The question of whether the predominant races of a region maintain their predominance year after year or periodically give way to other races, is of particular interest to investigators concerned with the development of new varieties because any such radical change is equivalent to a change in the pathogenicity of the rust. Evidence bearing on this question is available for at least two regions, North America and Australia, in which surveys for physiologic races have been conducted for many years. In North America, where data are available for a quarter of a century, several fluctuations have occurred in the relative abundance of particular races. During the period 1919 to about 1924, the two rather similar races 17 and 29 were more common than any other races (115, 142). From that time on they diminished in prevalence until during the early thirties they made up an inconsiderable part of the total isolates of races. In 1940 and 1941 they again increased to such an extent that they almost regained their earlier predominance (202). About 1925 a group of three similar races, 3, 18 and 36, assumed a preponderance (142, 199, 232) that was maintained until shortly after 1930, since when they have been collected but rarely. In 1934 race 56, which was first collected in 1928 (163, 202), became rather suddenly the predominant race throughout the United States and Canada, a position it has maintained up to the present time. The virtual disappearance of races 3, 18 and 36 is paralleled by that of races 21 and 49. Race 21 was collected commonly each year until about 1940, since when its occurrence has become a rarity; race 49 was one of the common races from 1927 to 1933 but has occurred only sporadically in recent years.

In Australia, from which records are available for the years 1922 to 1938 (239), there have been somewhat comparable though less striking fluctuations in the prevalence of particular races. From 1922 to 1928, races 43, 44, 45 and 46 were present in considerable abundance in most years. Since then races 44 and 46 have not been collected, and the other two have occurred only sporadically. On the other hand, race 34, collected the first time in 1926, became the most prevalent race two years later and has since shown a large predominance over other races.

Only occasionally has it been possible to give a satisfactory reason for the origin of new races or their rise to prominence. Waterhouse (237, 239) has furnished reasons for supposing that race 11, which occurred naturally for the first time in Australia in 1935, had its origin in aecia derived from a natural selfing of race 34, the predominant Australian race. In 1928, seven years before the discovery of race 11 in the field (233), he isolated that race and race 56 from aecia that arose most probably from a selfing of race 34 or possibly from a cross of that race with race 43. His conclusion that race 11 probably arose from a selfing of race 34 is supported by unpublished work by the present writers in which the selfing of a pure culture of race 34 produced uredial cultures of race 11 as well as race 34.

It has been suggested (190) that the origin of race 56 in America is possibly also connected with aecia on barberry. This race was first found in nature in Iowa, Kansas and Nebraska in 1928, some of the collections being obtained from uredia in close proximity to barberry bushes (163). It was not collected in the uredial stage in Canada until 1931; but it was first identified in September, 1927, from aecia on an artificially infected barberry turned over to the writers by J. H. Craigie (151). The occurrence of this race in both Canada and Australia in cultures derived from aecia is suggestive that its first appearance in the field may be attributed to natural infection from barberry. In regions where barberry is present, it is indeed likely that this shrub plays the chief part in the origin of new races.

Why one race rather than another should multiply and gain prominence is, in most cases, equally difficult to explain. In race 56 there is some evidence that its increase in prevalence was, at least in part, due to the susceptibility to it of the wheat variety Ceres which was widely grown for some years in the spring wheat areas of the United States (100, 190). The increased susceptibility that this variety showed as race 56 became more and more predominant is a good example of the practical importance that particular physiologic races may assume. It is, of course, obvious that the increase in the abundance of any physiologic race is conditional on the presence of susceptible hosts, but there are other factors that are also of importance. One of these is the length of the period of urediospore production. Physiologic races differ greatly in this respect. Some produce urediospores for only a short period before the development of teliospores sets in. Others, and these include the most prevalent races, remain for much longer periods in the uredial stage before they begin the formation of telia—a characteristic that obviously favors their spread in any season (94, 144). Races differ also in their ability to develop under adverse conditions of temperature (23, 96, 134), and they may also vary in ability to survive winter weather, a subject on which little is known at present.

Physiologic specialization in Puccinia graminis Avenae

In contrast to the large number of physiologic races in the variety *Tritici,* only 13, or possibly 14, races are known in the variety *Avenae.* Stakman, Levine and Bailey (194) in 1923 described four distinct physiologic races of oat stem rust. They inferred the existence of a fifth race which was later described by Bailey (8). These five races were distinguished by the infection types produced on three differential oat varieties, White Tartar, Richland and Joanette Strain. Only three of these races (races 1, 2 and 5) were collected in North America, where they, however, were widely distributed. Race 3 was found in rust collections from Sweden and South Africa, and race 4 occurred only in a collection from Sweden. Gordon and Bailey (73) collected all these races in Canada in 1925 as well as a sixth race (race 6). Two new races, race 7 (originally numbered 6) and race 8 (originally numbered 7), were reported by Waterhouse (234) from Australia in 1929. These two races were also found in Canada (74) about the same time, as was also a ninth race, race 9, which was first isolated there in 1930. Race 10 was discovered in the United States (26) in the same year, and race 11 was first collected in Germany in 1937 (83). Races 12 and 13 were first found in Canada (150). The latter originally occurred in cultures derived from artificially inoculated barberry and was collected in the field for the first time in 1944. A possible fourteenth race may be represented by a culture of stem rust collected in America on *Poa ampla* (49). As, however, it attacks several species of *Poa* and as all but one of the 17 varieties of cultivated oats tested proved immune, there is perhaps equal reason to regard it as a physiologic race of *Puccinia graminis Poae.*

When the distribution of the races is considered, it becomes evident that there are striking pathogenic differences in oat stem rust in different parts of the world. In North America races 1, 2 and 5 have predominated since the first studies on the specialization of this rust were made—a fact highly advantageous to plant breeders, as these races do not attack the varieties Richland and White Russian which have been used extensively as parents in breeding for resistance against oat stem rust. In Europe, as far as can be judged from the rather limited surveys conducted, other races with a wider range of pathogenicity are more common. Tedin (216) has reported the presence in Sweden of races 3, 4, 6, 7 and 8. Hassebrauk (81) studied six collections of rust from Germany and one from Bulgaria and found them to consist entirely of race 6. Later (83) he studied 13 collections from southern Germany and identified races 1, 2, 4, 6, 8 and 11. From his studies it would appear that the most common race is the highly virulent race 6 which attacks all oat varieties except a few produced recently that have not yet been distributed for cultivation. In South Africa only races 3 and 7 appear to have been reported (228), and in the Argentine the same two races are known to be present (227). In India races 3, 4, 6 and 7 have been identified (133), and in Australia the presence of races 1, 2, 3, 6, 7 and 8 has been established (239). Races 1 and 2 were by far the most common in Australia where they occurred annually from 1925 to 1938. They were also found in collections made in New Zealand. Races 3 and 7 were not uncommon in these two countries in certain years, but races 6 and 8 were rarely collected.

The question of whether the same races persist over a period of years in the same region is one of considerable practical importance, but it is a question that can be discussed only for regions in which yearly surveys have been conducted for a considerable time. Only in North America (the United States and Canada) and in Australia have annual surveys been carried out for periods long enough to warrant discussion. In North America races 1, 2 and 5 have maintained their predominance since surveys for races of oat stem rust were first undertaken, about 1921, and there is as yet no definite indication of their displacement by other races, although in 1942 and 1943 there was a notable increase in the prevalence of races 8, 10 and 11 (150, 200). In Australia (239) races 1 and 2 have been the predominant races throughout the period 1925–1938, with races 3 and 7 holding a rather distant secondary place. In 1938, the last year for which data are at hand, a considerable increase took place in the proportion of the last mentioned races—an increase that would, if maintained in succeeding years, make these races of much greater importance than previously. Despite these indications there is no conclusive evidence of great fluctuations in the prevalence of the various races of oat stem rust comparable at all with those already recorded for races of *P. graminis Tritici.*

Physiologic specialization in Puccinia graminis Secalis

One of the most essential qualities of good differential varieties is homozygosity of the plant material of each variety, which ensures uniformity of the rust reaction of the plants. In cereals that are self-pollinated it is easy by means of periodical selection to maintain the desired purity of each differential host. In a cross-pollinated crop, such as rye, the investigator of the specialization of its rusts is faced with the difficulty of securing lines with even a reasonable uniformity of reaction to rust, and, having once secured such a line, he encounters further difficulty in maintaining its purity.

Levine and Stakman (119), however, found that certain commercial varieties of rye reacted clearly enough to indicate that there were two and probably three distinct physiologic races of *Puccinia graminis Secalis.* One race possessed the ability to attack heavily the varieties Rosen, Swedish and Prolific. A second race attacked Rosen heavily, Prolific moderately and Swedish weakly; and a third race produced moderately heavy infection on Swedish and Prolific but weak infection on Rosen.

Later, Cotter and Levine (28) identified 14 physiologic races from 147 collections made in the United States, Canada, France, Scotland and Sweden. They used as differential hosts the varieties Rosen, Swedish, Prolific, Dakold and Colorless. The heterozygosity of the plant material necessitated the testing of large numbers of plants of each variety—an average of 200 plants being infected with each culture of the rust. A variety was considered resistant if more than 75% of the plants developed infection types 0, 1 or 2; mesothetic if less than 75% of the plants developed infection types 3 or 4, or when infection on all or most of the plants was type x; and susceptible when 75% or more of the plants showed types 3 or 4.

Of the 14 races identified, all were found in the United States except race 6 which was collected in Sweden. The bulk of the isolates from the United States, almost 60%, was made up of races 7 and 11. Collections from the other countries were too few in number to provide information on the predominance of any particular race.

Outside of the work reviewed above, the specialization of this rust has received but little attention. Verwoerd (228), however, states that two physiologic races of *P. graminis Secalis* are present in South Africa.

Physiologic specialization in Puccinia triticina

Mains and Jackson (125) in 1926 demonstrated the existence in *P. triticina* of 12 physiologic races which they distinguished by the reactions of 11 wheat varieties of which eight have since come to be generally accepted as differential hosts for this rust. Since then work on the determination of physiologic races has been undertaken in many countries, and new races have been described (7, 9, 21, 38, 105, 132, 171, 174, 175, 184, 186, 187, 230, 231, 236) in practically all regions where wheat is an economically important crop.

As there have been a greater number of separate and independent studies on the specialization of this than of any other cereal rust, it is not unnatural that a considerable confusion should have arisen with respect to the numbering of new races. Frequently investigators have assigned certain numbers to new races in ignorance of the fact that the same numbers had been used elsewhere for different races or that the same race had been described elsewhere under a different number. This confusion has, as far as possible, been clarified by periodical revision of the numbering of races described by individual investigators[3]. The last such revision contains 129 distinct races, but this is probably short of the actual number of races described up to the present time.

In this rust the number of physiologic races known to be present in different regions varies very considerably. In North America about 70 races have been collected up to the present, and in Europe a large number of races are known to exist. In India only six races were collected in the eight-year period 1930–37 (133), and in Australia only two races were identified from more than 1200 collections made during the period 1927–1938 (239).

The reasons for these striking differences in degree of specialization are not clear. Possibly the diversity of physiologic races in Europe is due to the presence there of susceptible species of the aecial host, *Thalictrum* spp., which occurs commonly in southeastern Europe and is often heavily rusted (171). Despite the occurrence of species of *Thalictrum* that have been shown to be susceptible in artificial infection studies, investigators have generally failed to prove that the aecia occurring in nature on these species are connected with *P. triticina* (39, 40, 140, 173, 183, 184, 218). D'Oliveira (156), however, has reported the presence under natural conditions of aecia of this rust in Portugal on *T. flavum* var. *glaucum*. With reference to North America, Arthur (5) states that no field collections of aecia have been identified with *P. triticina* but ventures the opinion that such undoubtedly do occur. In India there is, according to Mehta (133), no evidence that native species of *Thalictrum* bear aecia of this rust. No information is available to the writers on the presence of species of *Thalictrum* in Australia, but, as only two races of leaf rust are known to occur in that country, it would appear that an alternate host, if present, does not function actively. That it could so function was shown by Waterhouse (236) who infected two species of *Thalictrum* with the two Australian races and from the aecia so produced isolated these two races and two others previously unknown.

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[3] These revisions have been done by leading authorities on leaf rust in the United States. The most recent is the "Third Revision of the International Register of Physiologic Races of Leaf Rust of Wheat (*Puccinia rubigo-vera tritici*)", by C. O. Johnston, H. B. Humphrey, R. M. Caldwell and L. E. Compton. April, 1942. (Mimeographed for private distribution.)

It is difficult to decide whether or not there are any pronounced pathogenic differences between the leaf rust of wheat present in different regions of the world, partly owing to the absence in many of these regions of surveys conducted over periods of years and partly owing to frequent lack of agreement between the findings of different investigators working in the same geographic area. There are, however, indications that certain races, or races with certain pathogenic characteristics, tend to predominate in some geographic areas. Races 1 and 11, which do not attack the variety Malakof, occur most frequently along the Pacific coast of the United States and Canada (105, 125, 149), and the former of these races has been found frequently along the Atlantic seaboard. Neither one occurs commonly in the interior of the continent. In the Mississippi Valley races 9 and 13, which attack Malakof heavily, are common but not much more so than certain other races that do not attack this variety (24, 105). In Europe a great variety of races, some of which attack Malakof and some of which do not, have been identified in the interior regions, whereas in Great Britain, Holland and Italy the great majority of the races present are incapable of attacking this variety (175, 186, 187, 220, 241).

There is no evidence of any great changes from time to time in the prevalence of the various races of this rust that are in any way comparable to the fluctuations already reported for races of wheat stem-rust. Nevertheless new physiologic races appear now and then and gain wide distribution, as exemplified by the present prevalence in the United States and Canada of race 128 which was unknown until a few years ago.

Physiologic specialization in Puccinia dispersa (P. rubigo-vera Secalis)

Probably the first indication of the specialization of this rust is contained in a report by Mains (121) of the existence of two pathogenically distinct strains which he distinguished by the reaction of an inbred line of rye. This line was susceptible to one culture of the rust and resistant to another, thus indicating the presence of two physiologic races. An investigation into the specialization of this rust was also carried out by Gassner and Kirchhoff (60). On the rye varieties tested, the cultures that were studied showed some slight pathogenic differences that were, however, scarcely significant enough to permit the differentiation of physiologic races. Tests with wheat varieties showed that, despite their very high resistance to this rust, it was possible to detect symptomatic differences between cultures. Of the 10 cultures studied, one was distinguished by its inability to cause necrotic lesions on the leaves and was, therefore, considered sufficiently distinct pathogenically to be regarded as a different physiologic race. The same culture also differed from the others by its tendency towards early formation of teliospores on rye.

Further evidence on the specialization of this rust is submitted by Waterhouse (240). He observed susceptible and resistant reactions on one and the same leaf of a rye plant and was able, through the selfing of selected plants, to produce plants that indicated by their rust reactions the presence in Australia of at least two physiologic races.

Physiologic specialization in Puccinia glumarum

From 1925 to 1928 Rudorf (178) tested the reaction of a number of wheat varieties to *P. glumarum* in Germany and found that some of the varieties that had been classified by Hungerford and Owens (91) as resistant to this rust in America were susceptible in Germany. He concluded, therefore, that the stripe rust occurring on wheat in Europe differed pathogenically from that in America. This conclusion soon found support in the work of several investigators. Allison and Isenbeck (2) in 1930 demonstrated the presence of four physiologic races in European stripe rust collections on wheat; at about the same time in Germany, Gassner and Straib (63) reported the occurrence of two physiologic races, and Appel (3) was able to distinguish five races. Wilhelm (242) a few months later reported the isolation of five physiologic races from rust collected in Germany, France and Sweden.

As these investigators worked independently of each other, their respective physiologic races were identified by means of different groups of differential hosts. Subsequently Gassner and Straib (65, 67, 69) developed a group composed of 11 wheat varieties to which were added later (206, 211) supplementary varieties of wheat, barley and rye. By means of these differential hosts 47 physiologic races of stripe rust were identified by 1939 in Europe (211, 214), exclusive of several races described by Becker (12, 195). Of these 47 races, 37 were regarded as specific to wheat, four to barley, two to *Agropyron repens,* one to rye, one to *Hordeum murinum* and one to *Hordeum jubatum.* The specificity of these races, however, was not great enough to permit them to be classified as varieties of the rust in the sense used by Eriksson (41).

Though *P. glumarum* has been found in many parts of the world, its development is favored by a cool or moderate climate in which the summers are not excessively hot. The economic importance of stripe rust is probably greater in northern Europe than that of any other rust. It is for this reason that most of the work on the specialization of this rust has been done in Europe and particularly in Germany where it is widespread and most of the varieties commonly grown are susceptible to it (210).

In North America the rust is common and occasionally destructive along the Pacific coast and in some of the inter-mountain regions of the United States and in certain areas of Mexico (89, 90), but is largely restricted in its eastward spread to the areas immediately east of the Rocky Mountains (90, 145, 180, 181). Only a limited amount of work has been done on the specialization of stripe rust in America. Bever (14) in 1934 established the presence in Montana and Idaho of two physiologic races which he did not at that time classify as to number but later designated as races 19 and 28[4]. Newton and Johnson (145) found races 8 and 13 in rust collections from western Canada, the latter race, which resembled Bever's race 28, being the common race in that region. Previously, however, race 8 had been collected many times in Europe, but race 13 had not been found elsewhere.

In South America stripe rust is prevalent in Chile, the Argentine and Uruguay (89, 126, 179), and Straib (212) has recorded the presence there of four physiologic races (races 30, 37, 38 and 39) that differ pathogenically from any of the races found in Europe.

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[4] Private communication to the writers. The race 28 identified by Bever is described under that number by Stakman *et al.* (195) but is not identical with race 28 described by Straib (211).

On the continent of Asia work on the specialization of *Puccinia glumarum* has been reported by Mehta (133) in India and by Fang (48) in China. During the period 1932 to 1937 Mehta identified, from Indian collections on wheat and barley, races 13, 19, 20 and 31, as well as four new races designated as A, D, E and F. His results indicate that the physiologic races in India differ from those in Europe and America, although race 13 had been found previously in Canada and races 19 and 20 had been collected in Turkey and Bulgaria. Race 31 had previously been found only in adjacent Afghanistan. The predominant race in India was the new race A, races 31 and 19 being next in importance. The other races occurred only rarely. In China, Fang identified nine physiologic races from 43 collections made chiefly in the province of Yunnan. These races cannot be compared directly with any collected elsewhere, owing to the fact that he did not use several of the differential hosts now in common use as differential varieties. A striking feature of the Chinese races, which distinguishes them from most races collected in other countries, is their ability to attack the wheat variety Chinese 166 which is susceptible to only three of the 47 races described by Straib (211, 214).

It seems clear from the work done thus far that not only does this rust possess a high degree of specialization but that this specialization differs in the different regions in which the rust is prevalent. Thus, for example, race 7, which is the most common race in Germany (210), has not been found outside Europe; race 13, the common race in Canada, has not been reported elsewhere, except rarely in India; the races reported from South America appear to be restricted to that region; and the most common race in India has not been discovered anywhere else.

Physiologic specialization in Puccinia coronata Avenae

Hoerner (88) in 1919 established the presence of physiologic races within the *Avenae* variety of *P. coronata* through the infection types developed on two oat varieties (Ruakura and Green Russian) by means of which he was able to distinguish four physiologic races. Within the next ten years investigators in the United States, Canada and Germany proved that this rust comprised numerous pathogenically distinct strains. Popp (170) studied a number of rust collections made in Canada and distinguished four races through the reaction of three oat varieties. Parson (159) identified five races by the reaction of four varieties, and Murphy (137), using eight differential varieties, identified nine races. Peturson (167) reported the presence of eight physiologic races in Canada.

From the varieties which these investigators used for determining physiologic races, Murphy (138) selected 11 that have since come into general use in America and have been adopted in several other countries. By means of these he was able to identify 33 races from collections made in the United States, Canada and Mexico from 1927 to 1932. Using the same varieties Peturson (168) determined the presence in Canada of nine of these races and two additional ones not previously described. Through the use of the varieties selected by Murphy, at least 82 physiologic races have been identified up to the present time[5].

A comparison of the races identified in different parts of the world shows that specialization of the rust is somewhat dissimilar in widely separated regions. In North America 75 races have been determined, namely, races 1–39, 41, 45, 46, 48–54 and 57–82, the predominant races being 1, 2, 3, 4 and 6. In South America, Vallega (224) has demonstrated the presence of races 1 and 45 as well as two new races 55 and 56 which have not been found elsewhere. He found that race 1 was widely distributed and common in the Argentine (as it also is in North America) but that the other three races were rarely found despite their wider pathogenic range. In Australia, Waterhouse (240) has reported the presence of races 3, 6, 40 and 47. The first three are known in America but the last two have not been found outside of Australia. In England, Brown (18) has identified races 6, 42 and 44, and race 43 collected in Portugal. Race 6 occurs commonly in North America and, as shown above, was found also in Australia, but the other races had not been discovered elsewhere.

It is not possible to make any direct comparison of the races identified in continental Europe with those reported elsewhere, owing to the employment there of other groups of differential hosts. Frenzel (54) studied collections of crown rust made in Germany and adjacent countries and, by the use of nine differential hosts, including four that had been used by workers in America, was able to distinguish 33 physiologic races. Straib (209), in a study of

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[5] Information supplied by Mr. B. Peturson.

144 uredial collections made in Germany, identified 139 physiologic races and three additional ones from aecia on *Rhamnus cathartica.* These races he determined by the use of 15 differential varieties that included several not used by investigators in America. The greater number of races identified in Germany than in America does not necessarily prove that crown rust there is much more highly specialized, as it is well known that an increase in the number of differential hosts used is likely to increase the number of races determined. In fact, Kingsolver and Murphy (106) studied a group of 81 single-pustule isolates which, when identified by means of the American differential hosts, were resolved into 19 races but comprised no less than 46 races when identified by means of Straib's differential hosts. Moreover, some of the German races were identified by the use of somewhat finer gradations than are ordinarily employed by American workers.

In the absence of a direct comparison of European and American races, it is still possible to compare some of their characteristics because the German workers used certain of the American differential hosts. In this connection the reactions of the varieties Bond and Victoria are significant. In North America, Bond is susceptible, as far as is known, to only six (or 8.0%) of the 75 races thus far collected, whereas it is susceptible to 36 (or 25.4%) of Straib's 142 races. Similarly Victoria is moderately susceptible to four (or 5.3%) of the 75 American races but is moderately susceptible to 45 (or 31.7%) of the German races. Although Straib's results are based chiefly on collections made in Germany and represent only a two-year survey, the comparisons indicate nonetheless a pathogenic difference between European and North American strains of crown rust.

Physiologic specialization in Puccinia anomala

The existence of physiologic races in *P. anomala* was first shown by Mains (121) who found three varieties of barley, Oderbrucker, Featherstone and Horsford, highly resistant to one culture of the rust and susceptible to another, and thereby established the existence of two physiologic races. He later (122) developed a group of nine differential varieties by means of which he repeatedly identified these two races from rust collections made in the United States. Although only these two races were found in America, he obtained evidence of a third race in Australia through tests that demonstrated that certain barley varieties shown by Professor Waterhouse to be resistant in Australia were susceptible to the American races.

Brown (16, 17), in a study of rust collections of Canadian origin, identified four physiologic races by means of the reaction of six barley varieties; but, as all his varieties were different from those used by Mains, it is not possible to make any direct comparison among the two groups of races.

Hey (86), by means of yet another group of varieties, identified eight physiologic races from 17 rust collections made in Germany. Ronsdorf (176), using Hey's differential hosts and one additional barley variety, identified a ninth race, also from Germany, and later (177) described two additional races derived from an American collection, thus bringing the total number of races identified by means of Hey's differential hosts to eleven. She also tested five of the German races for their infection types on Mains' differential hosts and found them to behave like one and the same race, from which she concluded that the set of differential varieties developed by Hey was better adapted for the differentiation of physiologic races than those selected by Mains.

D'Oliveira (155), using seven of the ten hosts selected by Hey, the one added by Ronsdorf and two of those employed by Mains, described 11 new physiologic races from collections made in Great Britain, Portugal and Spain. In addition he described seven new races that arose as a result of the selfing (on *Ornithogalum umbellatum*) of two known races, one new race that arose from crosses between two known races, and one new race that originated in the mutation of a race previously collected in England. At least 30 physiologic races of *P. anomala* are, therefore, known, exclusive of those identified by Brown and Mains, through the use of other assortments of differential hosts. The hosts developed by Mains have been utilized by Waterhouse (238) in Australia for a study of the specilization of the rust in that country. The results indicated the presence of a race similar to though not identical with Mains' race 1.

Owing to the fact that different groups of differential hosts were used by North American and European workers, it is not possible to make any comparison between the races present in the two continents. Ronsdorf (177), however, ventured the opinion that the American races probably differed from those in Europe.

According to the work thus far done in Europe, it is probable that specialization in *P. anomala* is quite extensive. In this respect it is probably significant that d'Oliveira (155) did not isolate from collections made in England, Spain and Portugal any of the races collected by Hey or Ronsdorf in Germany. Moreover, he found only one race (race 12) that was distributed widely in both Great Britain and the Iberian peninsula; other races collected were 13, 14 and 15 in Great Britain, 18, 19, 20 and 21 in Portugal, and 16, 17, 21 and 22 in Spain.

Reaction of physiologic races to environment

It was early realized that each of the cereal rusts was best adapted to a particular environment. Stripe rust (*P. glumarum*) develops best in comparatively cool weather and flourishes in early summer in northern Europe and in other regions with a moderately cool growing season. Leaf rust of wheat (*P. triticina*) is adapted to somewhat higher temperatures, and stem rust (*P. graminis*) is favored by the comparatively high temperatures of mid-summer (162). Adaptations to conditions of moisture or of light are less obvious; in regard to stripe rust, which commonly develops in the fall and spring seasons, it is, however, possible that the abundant moisture and dull weather of those seasons are factors particularly favorable to this rust.

In view of these environmental adaptations, it is not surprising that the physiologic races of the various rusts should respond rather readily to the environment to which they are exposed in greenhouse studies. Each rust has its optimum conditions of temperature and perhaps also of light, and any marked deviation from this optimum is reflected in its development. In general, a rise in temperature shortens the incubation period—the time elapsing from infection to the outbreak of uredia (52, 61, 139, 160); but it may also have other effects. For example, in stripe rust, which requires cool weather for satisfactory growth, a high greenhouse temperature will render the host resistant and may even completely inhibit rust development (62, 145). Obviously this influence of temperature is of great importance in the determination of the physiologic races of this rust, as the infection types, by means of which races are determined, are strongly affected. The same conditions that influence the expression of this rust in the greenhouse will determine its behaviour in the field. It has been shown, for instance, that wheat varieties that are susceptible to it during the cool weather of early summer tend to develop a considerable amount of resistance on the advent of warm summer weather (13, 68, 109, 172, 215). Temperature conditions also have a pronounced effect on the distribution of stripe rust. It has been suggested (145) that in western Canada, where there is a limited spread of stripe rust each spring in an easterly direction from the foothills of the Rocky Mountains, the high day temperatures in the summer months check the growth of the rust and thereby tend to restrict its eastward distribution. In tropical or sub-tropical regions this rust thrives chiefly in the cooler conditions of high altitudes. In Kenya (British East Africa) it is prevented from spreading to low altitudes by the higher temperatures that prevail there (127). In India it survives the summer in mountainous districts and spreads down into the plains during the cooler weather of winter (130, 131, 132, 133).

The other cereal rusts, though somewhat less reactive to temperature, are sufficiently influenced to make it imperative that close attention be paid to that factor in the identification of physiologic races. It has been shown for stem rust of wheat (94), stem rust of oats (71, 72) and crown rust of oats (166) that physiologic races which under optimum conditions of temperature produce the X type of infection, may, at a higher temperature, produce the 4 type. In terms of host reaction this means that a mesothetic reaction may be changed to a susceptible one. The consequence might be that the same culture of rust would be identified as one physiologic race at a moderate temperature and as another race at a higher temperature. In the routine work of identifying physiologic races this sensitivity to environment is likely to result in different reactions on the part of certain host varieties at different seasons of the year (125, 234), unless important factors of the environment, such as temperature and light, are under effective control.

In leaf rust of wheat (*P. triticina*) and leaf rust of barley (*P. anomala*) the influence of temperature is complicated by the fact that the reaction of different host varieties is affected quite differently by this factor. It has been shown that the reaction of certain of the wheat varieties used as differential hosts for *P. triticina* tends to become increasingly susceptible with lower temperatures (64, 84), while that of some other varieties becomes increasingly susceptible with higher temperatures (84, 145). In some varieties the degree of change in reaction was found to depend on the physiologic race used (84). Much the same sort of relationship appears to exist between temperature and the reaction of certain of the barley differential varieties to *P. anomala* (86, 177).

Some of the other variable factors of the environment exert an influence on rust development second only to that of temperature. Deviation from optimum conditions of light should be avoided, as far as possible, in the determination of physiologic races, as deviations therefrom may alter considerably the rust reaction of a host. Subnormal conditions of light, in particular, generally tend to shift the reaction to rust in the direction of greater resistance (84, 94, 149, 161, 175) and to lengthen the period of incubation (50, 53, 85, 120, 134, 204). Excess of nitrogenous fertilizer influences rust reaction in the direction of increased susceptibility, whereas a deficiency of it or an excess of potash has the opposite effect (36, 57, 58, 59, 78, 158). Superabundance of soil moisture is reported to increase chlorosis and diminish the size of rust pustules (80). All the above-mentioned factors exert their influence most strongly on the reaction of plants that show a moderate degree of rust resistance, or a mesothetic reaction, while the reactions of plants that are immune, highly resistant or susceptible are usually affected less and often not at all.

Economic significance of physiologic races

The economic importance of physiologic specialization lies in the fact that the existence of many physiologic races in a rust confers on it a pathogenic versatility whereby the rust may attack a wide range of host varieties even if each individual race possesses only a narrow host range. It is not always possible for the plant breeder concerned with the production of resistant cereals to develop agronomically satisfactory varieties that are resistant to all the physiologic races of a rust. Where this is not possible, emphasis is usually placed on producing varieties resistant to the predominant races of the region in which the varieties are to be grown. If in this region there still exist physiologic races capable of attacking these varieties, the danger remains that these races, though perhaps previously unimportant, may assume greater prevalence and importance; and the more intensively these varieties are grown the greater is the danger that they may exert on the rust a selective influence favoring the increase of those races to which they are susceptible. It has indeed been maintained (76, 190) that such a selective action on the part of the wheat variety Ceres led to the rise to prominence in North America of race 56 of *P. graminis Tritici.*

Even when the plant breeder has been successful in producing varieties that seem to be resistant to all the physiologic races present, as appears to be the case in the production in the United States and Canada of varieties such as Thatcher, Pilot, Renown, *etc.*, there is still no guarantee that new physiologic races capable of attacking them may not arise. The appearance in the last few years in the United States of race 15 B—a biotype of race 15 capable of attacking these new varieties—is a potential threat to many of the recently produced rust-resistant wheats and an example of the emergence of a previously unknown strain of wheat stem rust (75, 76, 201).

The plant pathologist and the plant breeder responsible for developing rust-resistant varieties must face the fact that new physiologic races with pathogenic traits different from those of known races may arise at any time, particularly in regions where the presence of the alternate host enables crossing and selfing of existing races to take place. It must be admitted that, by virtue of the production of ever new physiologic races, the rust possesses a very considerable power of adjustment to its host varieties—a power that could obviously be greatly diminished by the eradication of the alternate hosts. Even, however, if the alternate hosts were destroyed, it is not safe to assume that new physiologic races could not arise. As far as can be seen at present, the price of security from rust damage is a continuous vigilance in the form of surveys to detect any pathogenic changes in the economically important rusts and unrelenting efforts to develop cereal varieties resistant to any virulent races that may arise.

HYBRIDIZATION IN CEREAL RUSTS

Nuclear phenomena

Before hybridization can be discussed intelligibly it is necessary that the nuclear phenomena involved in the different phases of the life cycle of the rust be understood. According to Arthur (4), the cytology of the rusts may be regarded to have commenced about 1880 with the observation by Schmitz (185) that the nuclei in the urediospores and mycelium of *Coleosporium Campanulae* (Pers.) Lev. were arranged in pairs. Later, Poirault and Raciborski (169) gave the paired nuclei the name "conjugate nuclei". Sappin-Trouffy (182) studied the behavior of the nuclei in many rust species. He established that the pycniospores and the hyphae from which they develop are uninucleate, but that the aeciospores, urediospores and the mycelia arising from them are binucleate. The binucleate condition persists in the immature teliospores; but in the mature teliospore a fusion of the two nuclei takes place. On the germination of the teliospore, four nuclei are produced as a result of two successive nuclear divisions of which one is a reduction division. These nuclei migrate into the developing sporidia (basidiospores) so that, when newly formed, the sporidia are uninucleate. Infection of the alternate host by the sporidia is soon followed by the development of the pycnia beneath which are formed aggregations of uninucleate mycelium that have been given the names "aecial primordia" (1) and "protoaecidia" (22), here referred to as "protoaecia". From these mycelial aggregations are later developed the binucleate aeciospores. The transition from the uninucleate to the binucleate condition was regarded by Blackman (15), Christman (25) and others as involving a sexual fertilization. The mechanism of this process, however, remained unknown until Craigie's discoveries that certain rusts were heterothallic (29, 31) and that the pycnia, which had previously been regarded as functionless, performed a definite function (30). He showed (32) that when pycniospores of *Puccinia graminis* and *P. Helianthi* Schw. are transferred from one pycnial pustule to a number of other pustules, aecia are formed beneath about one half of the pustules receiving the transfer of pycniospores; whereas, if no transfer is made no aecia are formed. Two important deductions may be made from his experiments: First, that the pycniospores are the bearers of one of each pair of the nuclei that become associated in the young aecium; second, that the pycnia may be classified into two different "sexual"[6] or intersterility groups. These groups he designated by the symbols (+) and (−).

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[6] Objection has been raised to the term "sexual" as applied to these groups on the ground that the pycnia and protoaecia are to be regarded as male and

Discovery of the function of the pycnia provided at once a means of bringing about the association of the nuclei of one physiologic race with those of another. In other words, it made possible the "crossing" of physiologic races.

Methods of crossing and selfing

Methods of crossing and selfing physiologic races of *Puccinia graminis* have been described (144, 152). In crossing two physiologic races, the pycnial exudate from a pustule (presumed to be of monosporidial origin) of race A is applied successively to several other similar pustules of race B. The resulting aeciospores contain one nucleus of race A contributed by a pycniospore and one of race B contributed by the uninucleate mycelium at the base of the pycnial pustule. The process by which the pycniospore nucleus reaches its destination in the young aecium has been discussed by Craigie (33) and Buller (22). The selfing of a physiologic race involves merely the intermixing of the pycnial exudate of pustules of the same race to bring about the various possible nuclear combinations.

The great bulk of the work on hybridization in the rusts has been carried out with the economically important species *Puccinia graminis* Pers. which, though regarded as a morphological unit, is composed of several physiologically distinct subspecies originally designated by Eriksson (41) as *formae speciales* but here referred to as varieties. Each of the three varieties that are pathogenic to cereals (*P. graminis Tritici, P. graminis Avenae, P. graminis Secalis*) comprise, in turn, a number of physiologic races. In crossing strains of *P. graminis,* two kinds of crosses are therefore possible, crosses between the different physiologic races of a single variety (intra-varietal crosses) and crosses between physiologic races of one variety and those of another variety (inter-varietal crosses). In the present paper these two phases of hybridization will be discussed separately.

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female organs, respectively. As these organs are present in the haploid thalli of both (+) and (–) groups, it is argued that each thallus is hermaphroditic but self-sterile. As, however, the pycniospores of one group can fertilize the protoaecia of the other, the two groups are cross-fertile but the cross fertility is dissociated from sex, the factors for (+) and (–) therefore being interfertility factors. Functionally, the (+) and (–) elements act as gametes, for in a "cross" between two physiologic races they bring together the haploid nuclei of the two "parents" to form a dikaryotic and finally a truly diploid condition.

Investigators have, in general, kept in mind two main objectives, first, to compare the pathogenic characteristics of the hybrid (F_1) race or races with those of the parental rust races used in the cross, and second, to study the inheritance, in the first and in subsequent generations, of any observable characteristics of the rust. To accomplish these aims it is necessary to have an exact knowledge of pathogenic and other characteristics of the parental strains; and the parental strains should, furthermore, be kept available for comparison with the hybrid rusts. When a study is to be made of the inheritance of rust characteristics in later generations, the hybrid rusts must be grown in pure culture in the uredial stage and then allowed to complete the life cycle by the production of teliospores, which must be induced to germinate and infect barberry (*Berberis vulgaris* L.), the alternate host of stem rust. In the reduction division that occurs during the germination of the teliospore, segregation of genes takes place so that these become variously distributed among the haploid sporidia and consequently also among the haploid infections produced by these sporidia on leaves of the barberry. After the haploid infections have produced pycnial exudate, the process of selfing is carried out by intermixing the pycniospore-bearing exudate of the different haploid pustules. By this process new associations of nuclei (and, therefore, of genes) are brought about in the binucleate aeciospores of the new generation of rust. Study of the strains of rust appearing in the new generation is primarily a study of the effect of these nuclear associations. As mass cultures established from numerous aecia would produce a mixture comprising several strains of rust, investigators usually initiate cultures from individual pustules or preferably from individual aecial cups. It has been established (152) that approximately 95% of cultures started from individual aecia contain, each, a single physiologic race.

Rust characteristics suitable for study

Before a discussion of experimental results is begun it is advisable to mention some of the chief rust characteristics that have proved amenable to study. It is obvious that no genetical studies can be conducted with an organism unless it possesses some properties that may be regarded as characters. In *Puccinia graminis,* morphologic characters are not available for study because the spores (aeciospores, urediospores, teliospores) of the various physiologic races within a variety, and indeed also the spores of the different varieties, appear to be virtually identical. The physiologic races are based entirely on physiological, *i.e.*, pathogenic, differences. Nevertheless, the pathogenic criteria—the infection types—are visible phenomena and are characteristic for each physiologic race. Thus, race 14 of *P. graminis Tritici* produces on seedling leaves of the variety Marquis a minute rust sorus about the size of a pinhead, surrounded by a halo of necrotic tissue (type 1); whereas race 15 produces on the same host a large elongated sorus that may attain a length of half a centimeter or more and is not surrounded by necrosis (type 4). By selfing these races it is possible to discover whether or not they are homozygous for the respective characters. If they prove homozygous—that is, if all the progeny of the selfing study reproduces the character in question without appreciable variation—it is possible to cross the two races with good prospects of learning whether one of these infection types is dominant over the other or whether the hybrid rust is characterized by some entirely different type of infection. In such a cross all the pathogenic differences shown by the two physiologic races may be studied one by one. For example, the two races mentioned above differ also by the infection types produced on the wheat varieties Kanred, Kota and Vernal, on which race 14 produces minute necrotic spots or small pustules of the 1- type whereas race 15 forms large sori of types 3 and 4.

Spore color, particularly the color of the urediospores, is another character that has proved amenable to study. Though some differences of color do exist in the uredial stage of stem rust collected in nature (143, 234), these are usually too slight to afford a satisfactory basis for study. Strains of strikingly abnormal color do, however, occasionally arise, presumably by mutation. Newton and Johnson (143) reported two such deviations from the normal red color of the uredia. One of these consisted in the production, among the red uredia of *P. gr. Tritici* race 9, of a few sori of orange color from which originated an orange strain of this race. The other consisted in the development from aecia of a strain of race 36 with greyish-brown uredia. Stakman, Levine and Cotter (196) likewise developed from aecia three strains of *P. gr. Tritici* race 58 described as Argus Brown, Dull Auburn and Mars Yellow[7]. Waterhouse

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[7] Ridgway's Color Standards and Color Nomenclature.

(234) and Gordon (72) obtained strains of *P. graminis Avenae* with orange uredia from aecia on barberries inoculated with telia gathered in the field. As the uredial color of these strains is readily distinguishable from that of normal rust, the color changes provided investigators with visible characters whose inheritance could be studied in selfing and crossing experiments.

Deviation from the normal in color of the aecia has also been noted. Aecia of strains with red or orange urediospores were orange in color, while aecia of strains with greyish-brown urediospores were a pale yellow (153.) In *P. graminis Agrostidis,* white pycnia and aecia have been found (27).

The fact that different physiologic races of the same rust vary greatly in their readiness to produce teliospores—some producing them rapidly after a short interval in the uredial stage, others only slowly after a long interval—is another rust characteristic that may be subjected to inheritance studies.

Although the above characteristics all pertain to the binucleate or dikaryotic phase of the rust, it does not follow that there are no characters of the haploid phase that can be made subject to inheritance studies. Morphologically, however, the pycnia of different races appear to be much alike, and consequently the only haploid characters that thus far have been studied are certain abnormalities that have arisen, such as the production in some physiologic races of white (chlorotic or necrotic) haploid infections with few pycnia and little or no capacity to develop aecia.

Selfing of physiologic races

Among the first attempts at genetic studies in the rusts was the selfing by Newton, Johnson and Brown (152) of several physiologic races of *P. graminis Tritici.* Of eight physiologic races studied, only one proved to be homozygous for all of the various pathogenic characteristics expressed on the 12 differential wheat varieties used for determining physiologic races. This and later studies (98) have shown that the majority of physiologic races of this rust are heterozygous for pathogenic characters. Frequently, however, a race heterozygous for certain pathogenic characters is homozygous for others—which shows that more than one pair of genetic factors are operative. Thus a race may be homozygous for the infection types developed on all but two or three differential hosts, and yet the heterozygosity for the infection types on these two or three varieties may lead to the production of several physiologic races in the progeny of a selfing.

Selfing studies soon produced information indicating the dominance or recessiveness of certain infection types. For example, races which do not rust the variety Kanred (0 type of infection) fall into two groups (98). Some produce, when selfed, only races that do not rust Kanred; that is, they are homozygous for that character. Others produce, in addition to such races, other races that rust it heavily (4 type of infection)—a fact indicating that inability to rust this variety (0 type) is dominant to the ability to rust it heavily (4 type). It follows that races phenotypically alike are not necessarily alike genotypically; or, in other words, two different cultures of the same race do not necessarily, when selfed, produce identical progeny. An example is afforded by the occurrence in the progeny of a cross between races 9 and 52 of two genetically distinct types of race 15, one of which was homozygous, producing only race 15 when selfed, whereas the other was heterozygous and produced a progeny consisting of races 15 and 52 (103).

Little work has been done in rusts other than *Puccinia graminis,* but it is likely that a heterozygous condition also exists commonly in physiologic races of other cereal rusts. In *Puccinia anomala,* d'Oliveira (155) has demonstrated the heterozygosity of three physiologic races, one of which, when selfed, produced 6 different races. Similarly Murphy (138) proved the heterozygosity of race 2 of *Puccinia coronata Avenae* by infecting *Rhamnus* spp. with it and re-isolating six physiologic races from the aecia.

Crossing studies with physiologic races of Puccinia graminis

Inheritance of urediospore color. Almost all samples of stem rust collected in nature have red urediospores, and most samples are homozygous for this character. Occasional samples of stem rust, however, produce, when selfed, strains with urediospores of atypical color (72, 143). It is the discovery of such strains of rust that has made possible a study of the inheritance of urediospore color.

Crosses between two physiologic races of *P. graminis Tritici* with urediospores of atypical color have been reported (103, 153). One of these races had orange urediospores with bright yellow or orange pigment in the cytoplasm but no dark pigment in the spore wall.

The other race had greyish brown urediospores that showed little if any orange pigment in the cytoplasm but contained a pigmented spore wall. Rust of the normal (red) color contains both pigments. Crosses between the two races of atypical color produced rust with urediospores of the normal red color. Selfing of the hybrid rust produced a progeny with urediospores of four distinct colors—red, orange, greyish-brown and white. The size of the color classes suggested a 9 : 3 : 3 : 1 ratio. According to this ratio, the inheritance of urediospore color could be explained by assuming the presence of two pairs of color factors, GG governing pigmentation of the spore wall and YY governing that of the cytoplasm, both of which are present in spores of normal red color. The genetic constitution of the greyish-brown spores in which cytoplasmic pigment was absent would then be GG yy; and that of the orange spores lacking the spore-wall pigment would be gg YY. The association of the two gametes in F_1 would produce the constitution GgYy which would account for the ratio 9 red, 3 orange, 3 greyish-brown and 1 white. The white spores, thus encountered in stem rust for the first time, appeared to lack pigmentation in either spore wall or cytoplasm, and presumably possessed the constitution ggyy.

The urediospores of the atypical strains were not the only spore-forms of these strains showing differences in color from normal rust. Greyish-brown and white strains produced aecia that were pale yellow or almost white in appearance with aeciospores lacking orange pigments in the cytoplasm. Teliospores of greyish-brown strains resembled those of red strains, owing to the presence of dark pigment in the spore walls, but teliospores of the orange and the white strains were distinctly paler than normal, owing to deficiency of the spore-wall pigment.

Although in the cross discussed above there is evidence for a simple two-factor scheme of color inheritance, it does not necessarily follow that the same two pairs of factors apply to all color inheritance in stem rust. The existence of minute variation in the color of normal, red rust collected in nature (55, 234), and the occasional occurrence, in hybridization work, of different shades of orange or of greyish-brown color, would suggest the existence of a number of alleles responsible for urediospore color.

Apart from the crosses described above, the only other crosses between strains of rust differing in color that have thus far been reported are those between races of *P. graminis Avenae* with red and orange urediospores (99). In F_1, red color proved dominant to orange. Information not yet published suggests a distribution of 3 red to 1 orange in the F_2 generation.

Inheritance of pathogenic characters. As indicated in the above section on the selfing of physiologic races, it is necessary, if intelligible results on the genetics of pathogenicity are to be obtained, to deal at any one time with a pair of contrasted pathogenic characters on a given host variety. In the cross race 9 x race 36 of *P. graminis Tritici* (98, 153), the contrasted characters of which the inheritance may be studied are given below together with the infection types produced by the hybrid rust:

		Infection types on host varieties				
		Kanred	Arnautka	Mindum	Spelmars	Vernal
(Parents)	Race 9	0	4	4	4	4
	Race 36	4	1	1	1	0
(Hybrid rust)	Race 17	0	4	4	4	0

If one considers first the contrasted pair of characters on the variety Kanred, the question to be decided by means of the cross is what infection type the hybrid rust produces on this variety. Each time the cross was made it was found that the hybrid rust was unable to rust Kanred, *i.e.*, the infection type was 0. The 0 type was, therefore, obviously dominant to the 4 type. On the varieties Arnautka, Mindum and Spelmars, the hybrid rust produced large sori (4 type), which showed that the 4 type was dominant to the 1 type (small sori). On the variety Vernal it was found that the hybrid rust produced a 0 type of infection. The 0 type produced on Vernal by race 36 was consequently dominant to the 4 type produced by race 9. Further work (98) has shown dominance and recessiveness of the same characters in crosses between other races, a fact indicating that these results are not peculiar to crosses between these two races but are of general, though not necessarily universal, occurrence in crosses between races of wheat stem rust.

Selfing studies with the F_1 hybrid rust (race 17) have shown that on the variety Kanred the 0 type, which was dominant in F_1, reappears in F_2 about three times as often as the recessive 4 type, thus indicating that a single pair of factors is operative. Similarly, on the durum varieties Arnautka, Mindum and Spelmars, the dominant 4 type occurs in F_2 about three times as frequently as the recessive 1 type, suggesting again a single pair of alleles. With respect to the variety Vernal, however, a different situation exists. In F_2, the dominant character, the 0 type, appeared about 18 times as frequently as the contrasting recessive character, a distribution suggesting that at least two pairs of factors are operative. As the factors governing these characters appeared to segregate and recombine at random, it was possible for several physiologic races to appear in the F_2 generation. On the assumption that the pathogenic behaviour of the rust is governed by one-factor pairs for Kanred and the durums Arnautka, Mindum and Spelmars, and by a two-factor pair for Vernal, it was expected that the F_2 generation would be composed of races 1, 9, 11, 15, 17, 36, 52 and 57. Actually all these races did occur in the F_2 generation and comprised the bulk of it. Four additional races, however, appeared, namely, races 29, 32, 85 and 110. As each of these four races resembles one or another of those in the first-mentioned group, they were regarded as merely variants and grouped with the races they resembled, *i.e.*, race 29 was considered a variant of race 17, race 32 of race 11, race 85 of race 9, and race 110 of race 15. The actual and theoretical distributions in F_2 are shown below:

	Race	17	11	1	36	9	57	15	52
Distribution	Theor.	171.4	57.1	57.1	19.0	11.4	3.8	3.8	1.3
	Actual	165[a]	64[b]	47	31	11[c]	4	2[d]	1

[a] Includes 6 cultures of the related race 29.
[b] " 5 " " " " " 32.
[c] " 6 " " " " " 85.
[d] " 1 " " " " " 110.

This study shows that Mendelian laws are operative in the inheritance of the pathogenic characteristics of stem rust of wheat, and indicates, furthermore, that an understanding of the way in which these characteristics are inherited can lead to a considerable amount of prediction as to which physiologic races may be expected to arise from crosses between known races.

In stem rust of oats (*P. graminis Avenae*) it has been shown, likewise, that certain pathogenic characters are dominant to others (99), and it is indeed probable that the inheritance of pathogenicity follows much the same pattern as in wheat stem rust.

Cytoplasmic inheritance. The pathogenic characters discussed above, as already stated, appear to be inherited according to Mendelian laws. It does not follow, however, that all pathogenic traits of stem rust are thus inherited. In certain crosses between physiologic races of wheat stem rust there is evidence that some pathogenic characters are inherited through the medium of the cytoplasm of the maternal parent race, *i.e.*, the race receiving a transfer of pycnial exudate. In crosses in which this type of inheritance is manifested, the pathogenic characteristics of the hybrid rusts arising from opposite sides of reciprocal crosses are not identical. Hence in the cross race 14 × race 36, the hybrid rust arising from the race –14 side of the cross conformed exactly to race 14, whereas the hybrid rust originating from the race –36 side of the cross was identified as race 88. The only difference between the reciprocal hybrid rusts was that, in the F_1 progeny from the race –14 side, the infection on Marquis was type 1 (as in the race –14 parent), whereas in the progeny arising from the reciprocal cross it was type X (as if influenced by the type 4 produced by the race –36 parent). The hybrid rusts arising from the reciprocal crosses were selfed to determine how far this cytoplasmic influence persisted in F_2 and F_3. The results (103, 144) showed a definite persistence in these generations of the infection types displayed on Marquis in F_1, a condition that would be expected if the original phenomenon were due to cytoplasmic influence.

A similar and even more clear-cut case of cytoplasmic inheritance has occurred in *P. graminis Avenae* in crosses between race 7 and race 11[8] (99). Race 7 produces on the variety Sevnothree a 4 type of infection (large sori), while race 11 produces a 1 type (small sori). The races arising from the race –7 side of a cross resemble race 7 in that they produce the 4 type on Sevnothree, while the races arising from the race –11 side resemble race 11 in that they produce the 1 type on this variety. This situation holds true not only for all crosses between race 7 and race 11 but apparently also for all crosses in which the two parent races differ in their infection types on Sevnothree. Consequently the same physiologic race never arises from a cross and its reciprocal cross.

This phenomenon does not seem capable of explanation on a purely chromosomal basis, but can be readily explained if it is assumed that the pycniospore nucleus of the paternal race reaches the protoaecium of the maternal race unaccompanied by its cytoplasm, which, in any case, is very limited on account of the minute

[8] Race 11 was originally numbered 10*a*.

size of the pycniospore. Each hybrid aeciospore would then receive from the paternal race only a nucleus, but from the maternal race a nucleus plus cytoplasm. This explanation, according to Lamb (110), "finds an entirely convincing cytological explanation" in his study of the initiation of the binucleate condition in *Puccinia Phragmitis* (Schum.) Körn.

In cereal rusts other than *P. graminis* only one record of possible cytoplasmic inheritance is known. D'Oliveira (155), in reciprocal crosses between races 13 and 23 of *Puccinia anomala,* obtained race 30 from the race -23 side of a cross but isolated two races, 30 and 18, from the reciprocal cross. Assuming that the infections were of monosporidial origin and that the nuclei of each haploid infection were identical, he attributes this result to differences in the cytoplasm among the hyphae that were diploidised.

Effect of inbreeding on rust characteristics. Each cereal rust possesses certain well defined characteristics of color, sporulation, *etc.,* which investigators regard as normal for the species; and, in rust collected in nature, deviation from a condition of normality is a rare occurrence. It was only when the component physiologic races of a rust came to be selfed—*i.e.,* when the alternate host was inoculated by a pure culture of a physiologic race followed by a close study of the uredial progeny arising from the aecia—that abnormal rust characteristics were observed in any considerable number. Though extremely rare in nature, deviation from the normal color of the uredia of *P. graminis* has been observed a number of times after the rust was induced to complete its life cycle by passage through its alternate host (72, 143, 196, 234).

Other abnormalities have also been noted, particularly when selected strains of *P. graminis Tritici* were selfed for several successive generations (97). The abnormalities observed were generally of a kind that under natural conditions would be detrimental to the survival of the rust, such as decrease in vigor of sporulation, *i.e.,* a tendency to form uredia with only a limited ability to rupture the epidermis, or decrease in pathogenic vigor as shown, for example, in the production of an X type of infection by cultures descended from strains that had appeared homozygous for the more vigorous 4 type of infection. Furthermore, certain strains originating through selfing have shown a greater sensitivity to high temperatures than strains collected in the field. Wheat varieties susceptible to such strains at ordinary greenhouse temperatures develop resistance toward them at temperatures above 80° F. (96).

Unusual characteristics encountered in selfing studies are not limited to the uredial stage of the rust. Abnormalities of the pycnial and aecial stages have been noted on several occasions. Cotter (27) observed the production on barberry by *P. graminis Agrostidis* of white pycnia with colorless exudate. Intermixing the exudate of these pycnia led to the formation of white aecia, whereas application to white pycnia of exudate from the normal yellow pycnia resulted in the formation of yellow aecia, as did also the application of exudate from white to yellow pycnia. Evidently, white aecial color is recessive to yellow.

In *P. graminis Tritici* the selfing of certain physiologic races has brought about results similar to though not identical with those just mentioned. Newton and Johnson (146) observed that in the selfing of a culture of race 21 two types of pustules were produced in about equal numbers on leaves of barberry. Pustules of one type were normal in appearance and produced aecia following the intermixing of the exudate. Pustules of the other type were almost white with either no pycnia or only a few scattered ones that produced exudate only after a considerable period. Formation of aecia in white pustules was rare and nearly always limited to rudimentary or otherwise atypical structures. A small proportion of the white pustules produced urediospores and teliospores after a prolonged period. By selfing studies and by crosses with another physiologic race, it was shown (101) that diploidisation of the mycelia of normal pustules by pycniospores from white pustules gave rise to physiologic races that produced white and normal pustules on the barberry in approximately equal numbers, whereas normal x normal matings produced normal rust, and white x white matings were sterile. It was assumed that a mutation affecting one of the two conjugate nuclei took place in the original culture of race 21 and that during meiotic divisions in the germinating teliospore the mutant factor is segregated so that half of the sporidia give rise to white and half to normal pustules.

A somewhat different effect that appears to be attributable to inbreeding is the loss of ability to produce aecia in certain strains of *P. graminis Tritici* (97, 146). This peculiarity appeared in several F_3 cultures of a cross between races 9 and 36. The pycnia of these cultures produced scanty exudate but formed no aecia as a result of either intermixing this exudate or application to them of the exudate of normal pycnia. In some of these cultures the loss of ability to produce aecia was associated with a rather marked tendency towards the production of urediospores and teliospores in old pycnial pustules. Some of these urediospores were found to be uninucleate (147).

The occurrence of uredia and telia of *Puccinia graminis* on barberry may possibly have some evolutionary significance in explaining the origin of short-cycled rusts from heteroecious long-cycled forms. Jackson (92) has postulated that microcyclic forms arise from the haploid generation of heteroecious eu-forms through the replacement of aecia by telia. Short-cycled species which appear to have thus originated are known as "correlated" species. The production of uredia and telia on barberry may perhaps be interpreted as evidence of a tendency on the part of *P. graminis* towards the formation of such a correlated species.

It is evident from the results given above that selfing of physiologic races, and particularly continued selfing of the same lines, brings to light characteristics unfavorable to the survival of the species. Such characteristics probably arise also under natural conditions but, not being adapted to survival, are soon eliminated so that they are infrequently encountered. In conclusion it may be stated that the development of abnormal strains of rust is not an inevitable consequence of inbreeding, as many inbred strains show no abnormalities. It is likely that abnormal characteristics are, in most cases, the result of recessive mutations that have taken place in the past history of the rust, the part played by selfing being that of segregating and recombining the mutant factors in a homozygous state under which condition their effects are manifested in various types of abnormalities.

Crosses between varieties of Puccinia graminis

The crossing studies already dealt with are concerned solely with intra-varietal crosses, *i.e.*, crosses between physiologic races of the same variety of stem rust. In such crosses interfertility of the different races appears to be complete. In inter-varietal crosses, on the other hand, some inter-sterility exists, the degree of which varies according to which varieties are crossed.

In crosses between the varieties *Tritici* and *Agrostidis,* Stakman *et al.* (196) found that when exudate from *Tritici* pycnia was transferred to *Agrostidis* pycnia no aecia were formed, but that when the transfer was made in the opposite direction aecia were formed in every case. Johnson *et al.* (102), in crosses between the same two varieties, transferred pycnial exudate from haploid *Agristidis* pustules to 14 haploid *Tritici* pustules, of which only one produced hybrid aecia. Transfers of exudate from *Tritici* pustules to 36 haploid *Agrostidis* pustules failed to produce any hybrid aecia. Crosses between the varieties *Tritici* and *Secalis,* however, led to the conclusion that little, if any, inter-sterility existed between these two varieties. In crosses between the varieties *Tritici* and *Avenae* (95) there was evidence of considerable intersterility.

One of the chief objects of making inter-varietal crosses is to discover the characteristic pathogenic properties of the hybrid rusts. Owing to several causes, this object has not always been successfully attained. In some crosses the great variety of pathogenic types occurring in the F_1 generation has made it difficult to interpret the results. In the crosses made by Stakman *et al.* (196) between *P. graminis Tritici* race 36 and *P. graminis Secalis* race 11, the F_1 progeny was resolved into eight physiologic races of *P. graminis Tritici,* namely, races 15, 21, 32, 36, 57, 67, 70 and 71, and two physiologic races of *P. graminis Secalis,* races 9 and 11. As far as can be judged from the pathogenicity of these races, only two of them (races 70 and 71 of *P. gr. Tritici*) could be considered at all intermediate between the *Tritici* and the *Secalis* varieties. The remainder of the races were either typical *Tritici* or typical *Secalis* races. These races may be hybrids in which *Tritici* or *Secalis* characters respectively predominate or, possibly, they may have resulted from accidental selfing of one or the other of the parental races. In crosses between the same two varieties, Johnson *et al.* (102) obtained an F_1 generation composed of four physiologic races identified as races 70, 104, 111 and 112 of *P. graminis Tritici.* Though described as races of wheat stem rust, they were remarkably weak in their pathogenicity on wheat varieties and even weaker in pathogenicity on rye. They lacked the ability to infect the wheat variety Acme which is attacked by nearly all other physiologic races of wheat stem-rust. Like both the parent varieties used in the crosses, they were capable of attacking barley. These four races, though distinguishable, are pathogenically similar and form, together with race 71 (mentioned above), a well defined group with pathogenic characters that may be considered more or less intermediate between those of the *Tritici* and *Secalis* varieties of stem rust. Even more narrowly limited to barley are certain hybrids from crosses between the varieties *Tritici* and *Secalis* reported by Levine, Cotter and Stakman (117).

A somewhat different result of *Secalis-Tritici* crosses is reported by Levine and Cotter (116) who described a hybrid rust capable of attacking both wheat and rye rather severely as well as barley. This rust, therefore, combines to a large degree the pathogenic powers of both parental varieties.

It seems probable that these two types of hybrids—the one possessing only to a limited degree the pathogenic powers of the parents, the other possessing a combination of their pathogenic properties—are both authentic and perhaps representative F_1 hybrids. In view of the great diversity of pathogenic types found in the parental rusts it is not surprising that considerable variation should likewise be found in their hybrid progeny.

Interpretation of the results of crosses between the varieties *Agrostidis* and *Tritici* is confronted with much the same difficulties as those already outlined in connection with *Tritici-Secalis* crosses. In crosses reported by Stakman *et al.* (196) the progeny, all of which arose on the *Tritici* side of the crosses, was composed of races that attacked wheat but not *Agrostis* species. At present it cannot be definitely decided whether the races are actual hybrids or the result of fortuitous selfing.

A *Tritici-Agrostidis* cross reported by Johnson *et al.* (102) produced different results. The progeny, which arose on the *Tritici* side of the cross, possessed to a very limited degree the pathogenic properties of both parental rusts. It was able to infect both wheat and *Agrostis* but only to a very slight extent. Like the *Tritici* parent rust it was capable of infecting barley, though not heavily.

A cross between the varieties *Tritici* and *Avenae* has been reported by Johnson and Newton (95). The hybrid, which originated on the *Tritici* side of the cross, possessed some of the pathogenic properties of both parental rusts. From the *Tritici* parent it inherited the ability to attack, though rather lightly, certain wheat and barley varieties. From the *Avenae* parent it inherited the capacity to attack certain varieties of oats and some of the grasses that are natural hosts of oat stem rust. The host range of the hybrid rust was, therefore, considerably wider than that of either parent rust; but this extension of host range was accomplished at the expense of pathogenic vigor, as the hybrid rust attacked any given host more lightly than did the parental rust of that host.

In general it would seem from the work thus far reported on inter-varietal hybridization in *Puccinia graminis* that hybrid rusts frequently show some of the pathogenic properties of both parental strains, but that this widening of host range is accompanied by a loss of pathogenic vigor towards any given host—a loss in some instances so great that the rust can find no really congenial host.

MUTATION

It is evident from the preceding discussion on hybridization that crossing and selfing of the different strains of a rust are capable of giving rise to much variation. The function of hybridization, however, is chiefly that of segregating and recombining the great variety of factors that already exist in the organism so that the greatest possible number of combinations may take place. Hybridization *per se* does not answer the question of how these factors came into being or how rapidly they change.

The stability of the genetic factors governing rust characteristics is a matter of interest because changes in these factors involve changes in the characteristics of the rust. If such changes are of frequent occurrence, it may be supposed that the adaptability of the rust to new conditions would be thereby increased.

During the relatively short period that has elapsed since cereal rusts came to be studied intensively, a considerable number of changes ascribable to mutation have been recorded. Changes affecting the color of a rust are perhaps the easiest to detect; and it is consequently not surprising that the first record of mutation in the cereal rusts should be of this type. Two deviations from the normal red color of urediospores of *P. graminis Tritici* were noted by Newton and Johnson (143)—one a change to orange, involving a loss of pigment in the spore wall, the other a change to grayish-brown, resulting from loss of yellow or orange pigment in the cytoplasm. The first occurred in a uredial culture under study in the greenhouse; the second was observed in uredia derived from aecia on artificially inoculated barberry, and was later (152) found to occur in uredia derived from the selfing of a pure culture of race 36. A mutation involving the production of yellow uredia was also reported by Waterhouse (234) in a culture of race 34 of wheat stem rust. Cotter and Levine (28) observed on two separate occasions in uredial cultures of *P. graminis Secalis* race 7, yellow uredia that gave rise to yellow strains of this race. In *P. graminis Avenae* Gordon (72) discovered orange strains of two physiologic races in uredial cultures obtained from aecia on a barberry inoculated with teliospore material gathered in the field. As grayish-brown and orange spore color is recessive to red, the above-mentioned grayish-brown and orange strains originating from aecia may perhaps be considered to have arisen from mutations that had taken place in the rust at some previous time. Possibly the same explanation may account for the origin of the brown and yellow strains of wheat stem rust discovered by Stakman *et al.* (196) in the progeny of *Tritici-Agrostidis* crosses.

Though some slight variation exists in uredial color of collections of stem rust gathered in the field (143, 234), radical departures from normal color are very rarely met with in nature. One such, however, a grayish-brown collection of oat stem rust is reported by Newton and Johnson (150), and another is possibly represented by a collection of race 40 of wheat stem rust with ochre colored uredia reported by Garbowski (55). It seems likely that striking deviation from normal spore color occurs among uredia derived from naturally infected barberries, but that such abnormal strains are not well adapted for survival and are thus infrequently encountered. It is probable, indeed, that the normal pigmentation of stem rust urediospores is a good protective agent against the ultra-violet light produced by the sun, for it has been shown (37) that spores of orange and white strains of wheat stem rust are more readily destroyed by ultra-violet light than those of grayish-brown or normal color.

Two records are known of what appear to be mutations involving both uredial color and pathogenicity. Johnston (104) described a field collection of *Puccinia triticina* differing pathogenically from known races of this rust and characterized by a long incubation period and by uredia lighter in color than those of other races. D'Oliveira (155) reported the production, in race 14 of *Puccinia* *anomala,* of an orange mutant differing pathogenically from the culture in which it appeared. Subsequent work showed at least one recurrence of the same mutation.

A number of mutations for pathogenic characters have been recorded in the cereal rusts. In *P. graminis Tritici,* mutations affecting pathogenicity have been described by Stakman, Levine and Cotter (196) who observed four separate mutations in the same physiologic race; and by Newton and Johnson (148) who reported an instance of a known physiologic race being gradually replaced by a previously unknown race. In *P. graminis Avenae* the last mentioned authors (150) encountered a somewhat similar case of the displacement of one physiologic race by another through a recurrence of the same mutation. In *P. triticina* Roberts (175) reported a mutation for pathogenicity in a culture under study in the greenhouse; and in *P. glumarum* Gassner and Straib (66) described a series of recurrent mutations resulting in identical mutants of greater infectivity than the original race from which they arose.

It will be evident from the instances cited above that mutation in cereal rusts cannot be regarded as an extremely rare phenomenon. Because it is usually difficult to prove that a mutation for pathogenicity has occurred, it is likely that many more such mutations have occurred than have been reported. Although mutations are not rare occurrences, it is probably not justifiable to conclude that isolated mutations occur at random in the various strains of a rust. It seems more likely that certain strains now and then develop a condition of instability which leads either to the production of several different mutations, as in the case reported by Stakman *et al.* (196), or, as seems to have happened more frequently, to the recurrence of the same mutations (28, 66, 148, 150, 155). The great majority of rust strains that have been kept in culture over any considerable period have given no evidence of mutability.

Not all mutations that take place in a rust are immediately observable. Selfing studies, in particular, have shown that there may be latent in a normal rust certain abnormal characteristics that come to light only when the rust is selfed. These are governed by recessive factors that presumably originate by mutation and become observable only when they are present in the homozygous condition. Although abnormal characteristics, such as those of color, are not well fitted for survival, it does not follow that all such unexpressed mutations cannot survive when brought to light. There exist in many strains of rust latent pathogenic potentialities that may reach expression in the new rust generation developing from the infected alternate host. These potentialities, perhaps originating in some genic changes of the past, may thus become actualities and possibly permanent rust characteristics. There can be no question that *Berberis vulgaris,* the alternate host of *Puccinia graminis,* plays its part in giving expression to the most diverse pathogenic characteristics. Extensive studies by Stakman *et al.* (197) showed that in aecial collections of *P. graminis Tritici* a different race was identified for every 4 collections, whereas in uredial collections made at random a different race was identified for every 100 collections. Even if only a fraction of the immensely greater variation evident in the rust derived from the barberry is originally due to mutation, there would still be ample reason to consider mutation a factor of great importance.

CONCLUSION

The 50 years that have elapsed since Eriksson's discovery of physiologic specialization in the cereal rusts have seen notable advances in man's understanding of these important pathogens. The discovery that the *formae speciales* or varieties, which were at first regarded as the ultimate units of specialization, were in turn made up of many specialized strains or physiologic races gave a great impetus to the study of the various rusts and, despite the fact that the existence of these physiologic races complicated the problem of developing rust resistant varieties of cereals, served also as a stimulus to greater efforts on the part of the breeders of rust resistant cereal varieties. The discovery of the existence of heterothallism in the rusts made possible studies on the crossing and selfing of the physiologic races of cereal rusts. Such studies have indicated that a great variety of physiologic races may be expected to arise by hybridization and have permitted a much more accurate evaluation than otherwise would have been possible of the significance of the alternate hosts in the production of physiologic races. These studies have also thrown considerable light on the genetics of the rust fungi and have shown that the inheritance of such rust characteristics as pathogenicity and spore color is subject to the same laws that have been found to govern inheritance in higher plants and animals.

The writers are indebted to Dr. J. H. Craigie and Mr. I. L. Conners for reading and criticizing the manuscript.

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THE BOTANICAL REVIEW

VOL. XII JULY, 1946 No. 7

PHYSIOLOGY OF CITRUS FRUITS IN STORAGE

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Although there are many species and many hybrids in the group of plants which produce our citrus fruits, only five species enter sufficiently into commerce to merit consideration in a treatise on the physiology of citrus fruits in storage. These five are: (a) the sweet orange (*Citrus sinensis* (L.) Osbeck); (b) mandarin and tangerine oranges (*Citrus reticulata* Blanco), *i.e.*, Satsuma, tangerine, King, Temple; (c) grapefruit (*Citrus paradisi* MacFad.); (d) lemons (*Citrus limon* (L.) Burm. f.); and (e) limes (*Citrus aurantifolia* (Christm.) Swingle). Mention should be made here of some of the more common commercial varieties grown in the United States of America because, as will be shown later, the variety may have a direct bearing upon its subsequent behavior in cold storage. The leading varieties of early orange in the United States are: Parson Brown, Hamlin, Washington Navel. Midseason varieties are: Pineapple, Jaffa. Most of the seedlings are also harvested along with the midseason budded varieties. The leading late variety is Valencia. Typical seedy varieties of grapefruit are Walters and Duncan. The Marsh (seedless or nearly so) is believed to have originated as a seedling. The pink-fleshed varieties of grapefruit are bud mutations of some of the pallid varieties. The Foster (pink-fleshed) was developed from the Walters and is a seedy type. There are also two popular seedless pink varieties. One of these, the Thompson (Pink Marsh), is a bud mutant of the Marsh; and the Ruby is a bud mutant of the Thompson. Two varieties of lemon, Eureka and Lisbon, are grown commercially in the United States. There are, likewise, two common varieties of lime. The small, pale yellow, Key or Mexican lime was probably introduced into the United States by the early Spanish settlers. The Tahiti (Persian) lime is much larger, almost the size of the ordinary lemon, and is marketed in the green stage.

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Citrus fruits are not held in cold storage to the same extent as are deciduous fruits. The latter must be removed from the tree before they become overripe. Citrus fruits, on the other hand, are usually "stored" on the tree until marketed. However, there are several exceptions to this statement. For example, when oranges and grapefruit are delivered to the metropolitan areas in quantities too large for immediate consumption they have to be held in storage. The Texas-grown grapefruit crop must be completely harvested before an established date (about May 1) in order to prevent infestation by the Mexican fruit fly. Adequate facilities for cold storage would help to extend the marketing period over the next two months. Another example of the use of cold storage facilities for holding citrus fruits is to be found in the lemon industry in California. The peak of lemon harvest in that state occurs during the late winter and early spring months, whereas the greatest demand for these fruits is usually in the summer. This may often necessitate a three- to six-month period of storage. Furthermore, the consumer demands medium-sized lemons which frequently necessitates picking and storing before they become too large. Other instances in which citrus fruits are subjected to at least the conditions of cold storage are the rail and steamship transportation of these fruits where the time of transit is sufficiently long to require refrigeration.

There is reason to believe that citrus fruits might be held in cold storage much more extensively in certain sections of the United States if facilities were available. In the Gulf States, for example, the commercial season for shipping oranges usually terminates about June 1. While it is true that up to this time oranges are held on the trees until marketed, many types of orange begin to "dry out" and regreen with the advent of warm weather, and could therefore be held much more advantageously in cold storage. This would serve to extend the marketing season well into the summer months because it has already been demonstrated by experiments in Florida that Valencia oranges may be stored until the latter part of July with 100% of the fruits remaining marketable (52). That there are problems that limit more extensive cold storage of citrus fruits is attested by the great amount of research being conducted on this subject in various parts of the world.

CHANGES OCCURRING DURING RIPENING

While this paper is concerned primarily with the physiology of citrus fruits in storage, it will be necessary occasionally to discuss certain phases of pre-storage physiology. The behavior of citrus fruits in storage may be greatly affected by certain cultural practices or by the manner in which fruits are handled after harvest. Likewise, since early and midseason oranges are usually "stored on the tree", so to speak, until a convenient time to ship them, it seems desirable to discuss briefly some phases of the physiology of citrus fruits during ripening.

Harding, Winston and Fisher (24) have described the changes occurring during the maturing of oranges on the tree and have presented a rather comprehensive review of the literature on the subject. Briefly these changes are as follows: There occurs a gradual increase in total soluble solids (principally sugars) and a decrease in acids, during ripening of oranges. At a certain stage of maturity the sugars and acids attain a proper balance and impart to the fruit a pleasantly tart or a sweet taste, and the fruit is considered ripe. Ascorbic acid (vitamin C), when expressed as milligrams per milliliter of juice, shows a slight decrease during ripening, this being more pronounced with Valencia oranges (late) than with early and midseason varieties. However, since ripening is accompanied by an increase in juice volume, the vitamin C is actually being diluted, and the loss is more apparent than real. Expressed on the basis of milligrams of ascorbic acid per individual fruit, there occurs an increase with ripening. Citrus fruits do not undergo a softening during ripening, as is characteristic of apple, peach, pear and banana. Other changes accompanying maturation are increases in size and weight, and a transition from green to yellow color in the rind. The changes in the constituents of the juice are very gradual when compared with those of deciduous fruits, so that the oranges may be held on the tree a considerable time before becoming overripe. In this connection Harding *et al.* state that: "Oranges left on the tree remain in good eating condition for a protracted period; however, if left too long granulation or drying out of the flesh, and an aged flavor, indicating senescence, eventually develop". Data presented by these authors indicated that early and midseason oranges left on the trees were considered by tasters to remain in the range of "pleasantly tart" to "sweet" or "very sweet" for two or three months, and there was at least one plot where the fruit was acceptable to the tasters for over six months during the cool part of the year. It should be borne in mind that citrus fruits do not ripen after removal from the tree. These fruits do not contain a "carbohydrate reserve", like the apple, pear or banana, which is converted into sugar upon ripening. All oranges are therefore "tree-ripened" if they are ripened at all.

Physiological changes during ripening of grapefruit, lemons and limes are very similar to those occurring in oranges.[2]

CHANGES OCCURRING IN CITRUS FRUITS AFTER HARVEST

The object of storing fresh fruits and vegetables at relatively low temperatures is quite obviously the retarding of the processes which cause these foods either to decay or to become less attractive, less palatable and less nutritious. The changes which occur rather rapidly in unrefrigerated citrus fruits may be classified into three groups: (*a*) parasitic or microbiological; (*b*) physical; and (*c*) chemical. Under the term "parasitic or microbiological" are included the several types of decay which may cause considerable loss in citrus fruits after they are harvested. The most common of these are blue mold and green mold, caused by *Penicillium italicum* and *P. digitatum,* the stem-end decays (*Phomopsis citri* and *Diplodia natalensis*), and the core rots, caused by species of *Alternaria.*

The most obvious physical change occurring in citrus fruits following harvest is the loss of water. This results in wilting or shriveling of the fruit with accompanying decrease in weight and volume. This type of change affects only the appearance of the fruit and renders it generally unattractive, although it may be accompanied by chemical or physiological changes which cause a loss of flavor and aroma.

Among the chemical changes occurring in citrus fruits after harvest are losses in sugars and acids, which are consumed during respiration. A reduction in these constituents causes the orange or grapefruit to become less palatable. Certain changes in the pectic compounds are responsible for the development of a dry or "mealy" consistency. The reduction in ascorbic acid directly affects the nutritive value of the fruit, although this loss in itself does not affect the flavor of the fruit. The loss of aroma, on the other hand, is due to chemical changes which are so minute that they are difficult to determine by chemical analysis.

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[2] Harding, P. L. and Fisher, D. F. Seasonal changes in Florida Grapefruit. U. S. Dept. Agr., Tech. Bul. 886. 1945.

CHANGES OCCURRING IN CITRUS FRUITS IN COLD STORAGE

That low temperatures are effective in prolonging the life of fresh fruits is quite obvious from the extensive use of cold storage facilities in this country. That lowering the storage temperature retards but does not always stop some of the changes is not always apparent, and the fact that certain processes may continue uninterruptedly even in cold storage is often known only to the research worker. Furthermore, at low temperatures certain disorders may become pronounced that do not occur in fruit held at higher temperatures.

The operator of a cold storage plant does not have to worry about the decay-producing fungi in citrus fruits for these organisms are fairly well arrested or retarded in their growth by low temperatures. After about a month at 50° F. a certain amount of blue mold, stem-end decay, or *Alternaria* core rot may develop, but the percentage of decay is not usually commercially significant. When citrus fruits are removed from cold storage, the decay-producing fungi resume their activity, unless a disinfectant treatment has been applied prior to storage. The longer the fruits are held in cold storage, the sooner they will decay following removal from storage.[3]

Physical changes, such as loss of weight and loss of water, are liable to continue in cold storage unless precautionary measures are employed. These changes are pronounced if the relative humidity of the storage atmosphere is too low and especially when there occurs a rapid movement of air of low relative humidity in storage. A relative humidity of 85% to 90% is usually recommended for the storage rooms in which citrus fruits are held, although a saturated atmosphere would be ideal from the standpoint of preventing water loss in the fruit. Since a saturated atmosphere is conducive to growth of molds in the storage rooms, it cannot be employed, and the commercial practice is to maintain as high a relative humidity as is practicable. The packer of citrus fruits also usually takes the additional precaution of lightly coating the fruits with wax to retard water loss.

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[3] Detailed discussion of the use of disinfectants for controlling decay in citrus fruits is not included in this paper because such a discussion seems to belong to the field of pathology. Mention has been made of the borax treatment which in the past has been almost universally employed in packing houses, but which in recent years is being largely abandoned because of its cumbersomeness. Of all the many new disinfectants that have been tested in Florida for control of decay in citrus and which are apparently free from hazards to health, the diphenyl compounds show the most promise and are perhaps the only ones that are equal to borax in effectiveness. These diphenyl compounds can be applied in an aqueous bath, they may be incorporated in a wax emulsion, or may be introduced into paper wrappers or box liners. Thiourea and several similar compounds have not been included in this discussion of disinfectants because their status has not yet been established.

Changes in chemical constituents of citrus fruits are a little more involved than physical changes and have been the subject of more extensive investigation. Some of these studies were begun as early as 1920. Hawkins and Magness (30, 31) reported that grapefruit showed a slight decrease in acids during a storage period of four months at 32° F. There were no appreciable changes in sugar. The loss in acids reported was rather slight, rarely amounting to more than 0.2 in percentage value. Somewhat later Stahl and Camp (52) verified these results insofar as acids are concerned, but claimed that reducing, hydrolyzable, and total sugars increased during storage. These results are a little difficult to understand, since mature citrus fruits contain no appreciable amount of starch that could be converted into sugar, as is true of some other fruits (apple, banana, *etc.*). It is possible, of course, that certain cellwall materials, such as pectins and hemicelluloses, could be hydrolyzed into free-reducing substances.

The bitter flavor in immature grapefruit is caused by naringin, a glucoside. Similar glucosides are present in other citrus fruits but not to the same extent as in grapefruit. It is interesting to note that several of the early investigators (30, 31) reported that the naringin content of Florida grapefruit decreased when the fruit was held in cold storage for four months. The claim was made that cold storage improved the flavor of grapefruit by causing a reduction of both acids and naringin.

Harvey and Rygg (25) have made a rather extensive study of the changes occurring in grapefruit rinds in an effort to throw light on the physiological disorders which manifest themselves in the rind tissues. In these studies samples of grapefruit were collected from the Corona and Fontana districts in California, where the harvest follows a relatively cool period, and from a district near Oasis, in the Coachella Valley, where the grapefruit crop is harvested following an extremely hot and dry summer. The fruits were stored for six weeks at 32°, 42° and 52° F. According to the results of these investigators, during the storage period there occurred a decrease in invert sugar in the rinds of the fruit from all sources. Reducing sugars increased at 42° and 52°, but the results for the 32° storage were inconsistent. Hydrogen-ion concentration increased at all temperatures and in both types of fruit.

There was a marked difference in the behavior of the fruit from the two areas in regard to changes in some of the other constituents of the rinds. Fruit from the Corona-Fontana district (harvest following a cool period) showed an increase in hydrolzable polysaccharides and a decrease in total soluble solids during storage. There were no great changes in these constituents in the fruit from the Oasis area. In the Corona-Fontana districts the naringin content of the rinds decreased at all temperatures, the greatest decrease occurring at 32° F. In the fruits from Oasis area, on the other hand, the naringin content increased at all temperatures, and the greatest increase took place at 32°. Hawkins, who reported a decrease in naringin content of Florida grapefruit in storage, collected the fruit from July 29 to December 9, but the fruits in which he reported changes in naringin content were collected during the cooler months (October, November and December). The fruits collected during July, August and September were not held at the lower temperatures. There is a strong suggestion in the reports from both California and Florida that naringin will decrease in stored grapefruit if harvested following relatively cool weather. However, Hawkins was referring to the juice, whereas Harvey and Rygg analyzed the grapefruit rind.

Additional contributions to the physiology of grapefruit in storage have been made by Rygg and Harvey (49). Samples of Marsh Seedless grapefruit were collected from three widely separated groves in California and stored at various temperatures. Total pectic substances in the albedo[4] showed a minimum early in the season for one lot and at midseason for the other two lots of fruit. The authors reported that variations of the total pectic substances in the albedo coincided with susceptibility to storage pitting, low pectic substances occurring at the time of low susceptibility. However, no causal relationship was postulated by the authors. Naringin content of the albedo of grapefruit from the field varied in much the same way as total pectic substances.

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[4] "Albedo" is the accepted term used for the spongy, white layer of tissue just beneath the thin, pigmented "flavedo" of the rind.

Oranges are like grapefruit in regard to changes in quality that occur in cold storage. It is the general consensus among investigators (52, 69), for instance, that early and midseason oranges undergo a slight loss of acids and show no significant changes in soluble solids during cold storage. This has been reported for oranges stored for two weeks to two months. Similar results have been reported for Valencia (late) oranges with, perhaps, one exception. Stahl and Camp (52) reported a slight increase in hydrolyzable and total sugars in Valencia oranges stored for four months. These workers also noted an increase in specific gravity, pH value, and total solids in the juice, and a decrease in per cent of juice in the fruit. The claim has been made for "Common" oranges in New South Wales (Australia) (14) that palatability of the juice and texture and color of the rind were improved by cold storage.

As in grapefruit Harvey and Rygg (26) have conducted studies of changes in the rinds of oranges in storage. Analyses were made on Washington Navel oranges, which are harvested during the winter months in California, and on Valencia oranges, the season of which begins in May and extends through the summer. Valencia oranges were stored at 33° and 53°, while the Navels were stored at 32°, 42° and 52° F. The period of storage was approximately seven weeks. According to these investigators the differences in results can be attributed more to differences in season than to differences in variety. However, despite the apparent varietal or seasonal differences there were several instances in which the behavior in storage was similar for the two varieties. Soluble solids in the rinds of both varieties decreased in storage, the rate of decrease being greater at the higher temperatures. There was an increase in hydrogen-ion concentration during storage at all temperatures. Determinations of the glucoside hesperidin were made on the rind of the Navel orange only, and it was found that this constituent also increased in storage.

Of considerable interest are the analyses conducted separately of the stem-end and blossom-end portions of the orange rinds. The investigators state that in the Washington Navel orange "the stem-end showed greater changes than the blossom-end in all substances under observation except active acidity". They likewise reported that in Valencia orange rinds the stem-end portion of the rind lost water and soluble solids more rapidly and contained higher hydrogen-ion concentration than did the blossom-end. The explanation of this increased physiological activity of the stem-end portion of the orange rind was to be found in the greater quantity of "albedo" tissue in this part of the fruit. The authors had previously reported the "albedo" tissue to be generally more "responsive" than the flavedo. These results are of considerable interest to the physiologist, and tend to confirm the observations of other investigators regarding differences between the two ends of the orange fruit. When Valencia oranges "regreen" in the spring, this renewed activity of the pigments always begins in the stem-end. Likewise "aging" and wilting in storage are always more pronounced at the stem-end.

In recent years considerable interest has been manifested in the vitamin C (ascorbic acid) content of oranges as well as the effect of prolonged storage on this constituent. According to reports from several sources, early, midseason and late oranges show no significant losses in ascorbic acid when held in storage for two weeks to three months. This has been reported by investigators in the United States (69), Australia (34) and in South Africa (23).

No great amount of work has been done on the storage of tangerines. Bratley (4) conducted some storage studies on the New York market in which these fruits were held for eight weeks at temperatures of 32°–33°, 36°–38°, 45°–48° and 53°–55° F. A marked loss of total acid and vitamin C was noted during storage at all temperatures, these losses being much greater at the higher temperatures.

The lemon, like the lime, has been used for both flavoring and therapeutic purposes for many years. It is of considerable economic importance, and the changes undergone by this fruit in storage should be of more than academic interest. Chemical analyses of California lemons stored at 32° to 60° F. give an idea of some of the changes occurring in these fruits in storage (43). Total sugar in both peel and pulp decreased in 15-weeks storage, the rate of decrease being greater at the higher temperatures. Analyses of the peel after 11 to 13 weeks showed increase in acidity and glucosides. Acetaldehyde tended to increase in storage, and this constituent was usually higher in fruit stored at 32°, 36° and 40° than at 50° or

60° F. Prolonged storage at 32° to 40° F. tended to reduce the reductase activity of the peel. In South African experiments (66) lemons were stored for four weeks at 40°, 45°, 50° and 55° F. When mature lemons still green in color were held at these temperatures, those at 50° F. developed more juice, soluble solids and acid than those held at the other temperatures. Studies made in California (27) indicate that during cold storage there is a reduction of the materials that are extractable by cold water or sulfurous acid. The compounds in question are no doubt those contained in the cell walls, such as pectins, hemicelluloses, *etc.* That there is no serious alteration in vitamin C content is indicated by the work of Delft (18) who stated that the antiscorbutic value of lemons remains unimpaired as long as the fruits remain in good condition.

RESPIRATION

Citrus fruits, like other fruits, continue to live for a while after removal from the tree. They respire, and during the process of respiration oxygen is absorbed, sugars and acids are oxidized, and water, carbon dioxide and heat are released. Generally speaking, the rate of respiration in citrus fruits is lower than in most other fruits and vegetables. It has been shown by several investigators (48, 22) that the rate of respiration of oranges at 32° F. falls within the range for that of apples and pears, which in turn are among the "low-respiring" deciduous fruits. The respiration rate for lemons and grapefruit is still lower than for oranges. When the temperature is raised, the rate of respiration increases much more rapidly in deciduous fruits than in citrus fruits.

In discussing respiration some mention should be made of the term "climacteric", which was first used by Kidd and West in connection with respiration of apples. After the setting of fruit, the respiration rate of various apple varieties is very high. The rate declines to a minimum at about the time most apple varieties are harvested, and then begins to rise again to a maximum. This peak in respiratory rate is termed the "climacteric". The respiration rate in apples and pears declines after the climacteric, and the fruits are said to be in senescence. A climacteric in respiration of oranges has been reported and will be discussed later (12).

A rather unique method for determining respiration has been developed by Harvey and Rygg (28, 49), and the method was found useful in predicting the storage life of grapefruit, lemons and oranges. The fruits were stored in a glass jar to which a manometer was attached. Shortly after the fruits were introduced into the jar a negative pressure was produced which reached a maximum when the supply of oxygen was exhausted. With continued carbon dioxide production the pressure soon increased at a fairly constant rate, eventually becoming positive again. A relationship was found to exist between the negative pressure and the potential storage life of the fruits. Longer duration and greater maximum of negative pressure indicated longer potential storage life of oranges, lemons and grapefruit. Also the longer the duration of negative pressure, the less susceptible were grapefruits to pitting in storage. The authors point out, however, that "while the relationship of the time of negative pressure to susceptibility to subsequent pitting holds roughly for fruit from an individual grove or group of trees, it does not hold when the behavior of fruit from one location is compared with that from another, perhaps many miles distant."

Accumulation of carbon dioxide in storage may have a direct effect upon the physiology of citrus fruits. It requires but a slight percentage of carbon dioxide in the coloring rooms to retard the rate of degreening of citrus fruits. In cold storage a certain amount of this gas may prolong the life of the fruit, but too high a percentage will have deleterious effects. This is discussed later.

PHYSIOLOGICAL DISORDERS OCCURRING IN COLD STORAGE

Thus far only slight mention has been made of the difficulties frequently encountered in attempting to hold citrus fruits in cold storage for extended periods. These fruits, like other subtropical fruits, are rather sensitive to low temperatures, with the result that sooner or later they may develop certain physiological disorders. These disorders may not only render the fruits unsightly and unpalatable, but also make them very susceptible to decay upon removal from cold storage. The great danger zone for development of these maladies is in the range of 32° to 40° F. The upper limit should be raised a little for lemons and limes. Before entering into the discussion of the occurrence of these low-temperature disorders, a description of some of the more common injuries will first be presented. These descriptions are based on information appearing in the U. S. Dept. Agr., Miscellaneous Publication No. 498 (47).

Pitting (*pox, chill spotting, brown spot, storage spot*). Pitting of citrus fruits consists of abruptly sunken spots in the rind. The spots, though not discolored at first, may later become slightly pink on grapefruit and eventually brown on both grapefruit and oranges. Early and midseason varieties of orange are more susceptible to pitting than are late-ripening varieties, and the Pineapple orange is the most susceptible variety. Although pitting may occur at the time of packing or in transit, it generally does not develop until after a storage period of four to six weeks. Softening often occurs under the pits and may lead to invasion by blue mold and other fungi. The pulp underneath usually has a tainted taste.

Pits on lemons usually appear as depressed areas in the rind from 1/16 to 1/2 inch in diameter, sometimes retaining their normal color but more often becoming light brown or dark brown, approaching black.

A similar type of rind breakdown in limes is not referred to as pitting because large areas are usually involved, more irregular in shape. These areas are sunken, having distinct and abrupt margins, and they vary in color from tan through rusty pink to deep brown. Although affected areas may occur on any part of the fruits, they are more common on the sides than at the stem and stylar ends. The flavor of the juice is not affected unless the fruits are severely spotted.

Watery breakdown. Citrus fruits affected with watery breakdown look very much like fruit that has been frozen. They are soft and spongy and have a water-soaked appearance. Both flesh and rind may be softened. The carpels are loosely attached to the inner part of the rind and, when a section of the rind is pressed, a watery substance oozes freely from the albedo.

Brown stain or scald of oranges. Brown stain or scald of oranges consists of a superficial and fairly uniform browning over relatively large areas of the rind, differing from pitting in the large areas affected and in not being sharply depressed. Also the color is seldom so dark as that of pitting.

Aging. Aging, sometimes found on oranges and grapefruit, becomes apparent after harvest. The rind around the stem button or upper part of the fruit becomes wilted or shriveled, with or without collapse of the rind tissues. Sometimes it is difficult to distinguish between pits and aged spots on grapefruit and oranges. It is customary in the United States to designate as aging any darkened and depressed spots in the rind in the vicinity of the stem.

Membranous stain or membranosis of lemons. Membranous stain of lemons is characterized by a browning or darkening of the membranes or carpellary walls between segments, sometimes affecting the central core tissues and inner tissues of the rind. There are no external symptoms. The irregular stained patches, varying in size from mere dots or streaks to large areas involving an entire membrane, are dark, yellowish-brown to sepia. This disorder is most easily detected by cutting the fruit lengthwise from button to nipple. The stain is seen on the divisions or membranes and not in the pulp cells or juice sacs. The juice is not perceptibly affected till membranous spots have occupied a large percentage of the membranes.

Albedo browning of lemons. Albedo browning takes the form of a discoloration of the white, spongy, inner part of the rind which is known as the albedo. The disease may be evident externally only as a slight darkening of the rind due to the discolored inner tissue showing through the surface layer. When the fruit is cut into, what is usually the white inner albedo portion is brown and sometimes shows a sort of gummy consistency.

Red blotch and peteca of lemons. There are several other rind blemishes of lemons which may occur in storage, although the fruits are first predisposed to these disorders by certain seasonal and weather conditions. Red blotch (adustiosis) is a superficial, scald-like browning of the surface layers of the rind. At first a light cinnamon brown, it may later darken into a chestnut brown.

Peteca resembles pitting, although the depressions are more gently rounded at the edges. The collapse begins with the albedo, and the outer layer of the rind sinks without first becoming discolored. The oil glands soon begin to darken, and in extreme cases the whole surface layer may collapse and become discolored.

Rind breakdown of oranges. Since pitting and aging of oranges are sometimes indistinguishable and since they are often both produced by the same storage condition, it is convenient to use the term "rind breakdown" to include both of these disorders in a discussion.

DEVELOPMENT OF PHYSIOLOGICAL DISORDERS IN COLD STORAGE

In studies with Florida grapefruit it was discovered as early as 1920 that the appearance of a breakdown or pitting of the rind was an undesirable result of holding this fruit too long in cold storage (30). The range of temperatures in which this disorder was found most liable to occur was 31° to 42° F., although it occured to some extent at temperatures as high as 58°. Instances have been cited by other workers (52) in which Silver Cluster (seedy) and Marsh Seedless grapefruit, stored for eight weeks at 32° and 37½°, have developed so much pitting as to render 35% to 40% of the fruit unmarketable. The effect of low temperature on pitting of grapefruit has been reported from Florida, Texas and California in the United States, and from Trinidad, Palestine, South Africa and Australia (20, 25, 34, 38, 52, 61, 63). As a rule, temperatures around 40° have been found to be much more conducive to pitting than higher or lower temperatures.

Watery breakdown, another low temperature injury, has also been reported on grapefruit stored at temperatures ranging from 31° to 42° F. However it is more often reported to occur on fruits held at 32° or lower and it is believed by some that in instances in which this disorder has been reported to occur at 32°, the temperature may have accidentally dropped below this for a short period. The disorder does not occur except after prolonged storage. Watery breakdown gives the fruit the appearance of having been frozen, and it is possible that claims for damage have been paid on fruits, in storage or transit, affected with watery breakdown because both parties involved believed the damage to be due to freezing. It will be shown later that there are several other factors that predispose citrus fruits to watery breakdown in cold storage.

Because of the danger of low temperature injuries to citrus fruits in cold storage, investigators for many years have been seeking the optimum temperature for storing these fruits. The extent of this search for an optimum temperature is obvious from the fact that reports contain the following temperatures or range of temperature at which grapefruit have been stored experimentally: 31°–32°, 35°, 37°, 37½°, 39°, 40°, 42°, 45°, 48°, 50°, 52°, 54°, 55°, 58°, 60°, 70°–80° and 86° F. The choice of an optimum temperature involves of necessity a compromise between the prevention of decay which occurs at relatively high temperatures and the prevention of physiological disorders occurring at low temperatures. Citrus fruits produced under irrigation in areas of insufficient rainfall (California, Texas, Spain, Italy, Palestine, Australia, all but the coastal areas of South Africa) are not so susceptible to stem-end decays as are the fruits grown in the regions having ample rainfall (Florida, South American countries, Japan, China, New Zealand). This probably accounts for the fact that investigators reporting on grapefruit from semi-arid regions designate 45°–55° as the optimum range of storage, whereas those in humid regions stipulate 32° to 37½°. The U. S. Department of Agriculture has taken this into consideration in its recommendations for the storage of grapefruit (48), which are: 45° to 55° for those produced in the sections of the country where stem-end decay is not a serious factor in holding fruit; and 32° for those grown in regions where this decay may shorten the storage life of citrus fruits.

The effect of low temperatures on stored oranges has been studied by research workers in many countries, and reports are available on these fruits produced in Australia, Brazil, India, Palestine, South Africa, Trinidad and the United States (2, 15, 34, 36, 38, 51, 52, 57, 59, 67, 75). The majority of these investigators have at one time or another reported the occurrence of low temperature injury, such as pitting of oranges when the fruits have been held for a month or more at temperatures below 40° F. In general, 36° to 40° has been found to be more conducive to pitting than higher or lower temperatures. In at least one instance oranges were stored in the range of 29°–32°, although this is much lower than is usually encountered in commercial storage houses. When the fruits have been held at or about 32° brown stain (scald) and watery breakdown have developed (26). In other words, a lower temperature is required to produce these two disorders than to produce pitting.

There has been at least one instance in which low temperatures are reported to have produced a bitter flavor in Navel oranges (75), in addition to the customary rind injuries, and there has been observed a type of spotting on this variety which appears at relatively high temperatures (50°–65° F.) and which differs from the usual low temperature breakdown of the rind (19, 64).

There is little agreement among investigators as to the optimum temperature for storing oranges. Taken the world over, the recommendations extend from 32° to 50° F. All of the investigators quoted have found one temperature or a very narrow range of temperatures that is more conducive to injury than others, but there has been no complete agreement on the critical temperature or range of temperatures. As with grapefruit, the temperature selected for storage of oranges must be a compromise between an effort to prevent decay at high temperatures and loss from rind injury at low temperatures. For long storage of oranges the U. S. Department of Agriculture has recommended 34° to 38° F. (48). Recommendation from other sources have varied somewhat from this. For shipments from Brazil 36° F. has been recommended (11), and for shipment from California (45) to the Orient, 36°–38° F. State workers in Florida recommend 37½° F. and those in California, 38°–40° F. In Texas 50° F. is recommended for Valencia oranges. General recommendations from South Africa and Australia seem to extend into the higher range *i.e.,* 37° to 50° F. Van der Plank *et al.* (in South Africa) (64) state that temperatures in the region of 50° to 55° F. are indicated for oranges that are under-colored, hard-textured or otherwise immature and that are resistant to decay, whereas lower temperatures (such as 40° F.) are more advantageous for fruits that are fully ripe or overripe or that are greatly subject to decay. The fact that most of the oranges grown in South Africa are produced in the irrigated section and are thus less liable to decay, may explain the tendency of investigators in this part of the world to lean toward the higher temperatures in their recommendation for storage of oranges. From the results of experiments conducted by the U. S. Department of Agriculture the ideal temperature range for commercial storage of oranges appears to be 34° to 38° F.

Physiological disorders occurring in lemons at low temperatures have been reported in the United States (9) and in South Africa (66). When held at 32° to 40° F. these fruits have developed injuries such as pitting, watery breakdown, scald, red blotch and membranous stain. As with other fruits, scald and watery breakdown occur more frequently at 32°. Pitting is usually more pronounced at 32° and 36°, and red blotch at 36° and 40°. Membranous stain is much worse on lemons stored at 40° than at higher or lower temperatures. Lemons are much more sensitive to low temperature injuries than are grapefruit and oranges, and, as a consequence, higher storage temperatures are usually recommended for them, *i.e.,* 55° to 58° This, of course, increases the danger of loss by decay although this is usually the lesser evil in areas where lemons are extensively produced. It has been found that green lemons stored at 50°–55° F. will develop as full color as those picked full-colored and stored at 40° or even better color (66).

Both Key and Tahiti limes are subject to rind breakdown in storage and transit (47, 68). These fruits are more subject to rind injury when held at 40° than at 45°, but if held long enough (eight weeks) at the higher temperature, they may still develop this disorder. Although this low temperature injury appears like pitting at first, it soon affects such large areas that it seems more like a distinct type of breakdown characteristic of only the limes. The damage is more severe on immature fruits than on mature ones, and limes stored in a dry atmosphere are more subject to breakdown than those held under moist conditions. Satisfactory storage of limes is usually obtained by holding at 45° to 48° F. with a relative humidity of 85% (48).

FACTORS AFFECTING THE INCIDENCE OF LOW TEMPERATURE INJURIES

Although the physiological disorders just described are usually referred to as low temperature injuries, there are certain other factors which, though not directly responsible for the injuries, certainly exert an influence on the fruits' susceptibility to low temperature breakdown. This may account in part at least for the great diversity in results that have been reported by research workers in different regions, not only in this country but in all parts of the world. Soil type, climate, cultural practices and position of the fruit on the tree are known to influence the subsequent storage life of citrus fruits. It has been observed in Florida that oranges and grapefruit produced in "low hammock" soils, which contain abundant moisture and are relatively high in organic matter, are more liable to breakdown in cold storage than are fruits grown on the "high pine lands" (39). Fruits produced in regions of abundant rainfall have a shorter storage life than those grown in semi-arid regions. Severity of pitting of grapefruit in storage has been shown to vary directly with the mean temperature for the five days preceding picking (49). It has been reported that grapefruit from trees receiving a high percentage of potash in the fertilizer has shown more physiological breakdown in storage than that from unfertilized trees (20). In South Africa grapefruits picked from the outside of the tree were found to be more susceptible to cold injury than those from the inside (65). Another factor in susceptibility to injury at low temperature is the variety of fruit involved. In the United States early and midseason oranges as a rule are more susceptible to pitting than the late ripening varieties, the Pineapple orange being the most susceptible variety. The Valencia orange is more susceptible to aging than to pitting. Similar results have been reported for grapefruit. A number of investigators have reported that seedy varieties may be more satisfactorily stored than the seedless ones (20, 31, 52).

Still another factor that may influence the life of citrus fruits in storage is the stage of maturity at the time of storing. It is physiological maturity rather than legal maturity that is meant here. An orange is legally mature when the ratio of solids to acids in the juice has reached a certain value, this value having been set by the officials of the State in which the fruit has been produced. By physiological maturity is meant the age of the fruit with reference to its respiratory climacteric. This is illustrated by experiments in Australia (12) in which oranges were picked a month before the normal time of picking, at the normal time and a month later. Measurement of the carbon dioxide output (at 40° F.) of all pickings during the whole storage period showed that the fruit from the first picking reached its climacteric in 60 days; the second in 30 days; and the third had just reached its climacteric at the time of picking. The time to reach 10% decay in each pick was 70 days after the climacteric. Thus the storage life of the three picks was 130, 100 and 70 days, respectively, and the life of the fruit in cold storage was prolonged by storing it as much in advance of the climacteric as possible, although the last date of holding would be the same for all lots. In this connection Stahl and Camp (52) state: "from the standpoint of metabolic changes, it is best to store slightly under-normal mature fruit . . .". They illustrate their point by presenting the results of storing Valencia oranges that were below normal, normal and above normal in maturity. At 32° storage it required six months for the first lot to undergo certain changes in acids and sugar that required only three months on the tree. The mature lots required four months to reach the same stage that was attained in two months on the tree, and the last lot underwent certain changes in two months that required one month on the tree.

There is evidence, on the other hand, that certain physiological disorders are more liable to appear in citrus fruits when they are stored during the stage of under-normal maturity. In experiments with Florida oranges Winston (74) harvested the fruit at three different stages, corresponding roughly to three stages of maturity, although the exact date of the respirational climacteric was not determined in these particular instances. He found that, in general, the amount of pitting, aging and wilting in Valencia oranges in storage decreased with each successive picking. Similar results have been obtained with Palestinian grapefruit stored in England (21). The early-picked fruits were found to be more susceptible to low temperature injury than fruits from midseason and late picks. It has been reported that membranous stain and red blotch are more liable to develop in stored green lemons than in fully colored ones (19, 66). It is possible that when the climacteric in citrus fruits occurs at low temperatures, there is a serious disturbance in the metabolic processes. Certain waste products may be formed more rapidly than they can be thrown off. The beneficial effects of pre-storage treatments, such as "curing" or exposure to carbon dioxide, may be due to their accelerating effect upon certain processes before the fruits are subjected to low temperatures.

If other conditions were equal and it came to a choice between storing oranges and grapefruit from early or late pickings, it seems more desirable to choose the latter. Holding the fruit on the tree would save cold storage charges, and, for a while at least, the fruit would be improving in quality. Stahl and Camp have advised against storing fruit that is over-mature because "it does not hold up well and takes on storage tastes rapidly". It is true that there is an end to all good things, but if the grower or shipper is familiar with his fruit he should be able to determine when the end is approaching. Some varieties of orange and grapefruit will last longer on the tree than others, and the same variety grown on sour orange rootstock will keep its quality on the tree longer than when budded on rough lemon stock. Grapefruits, being more acid than most oranges, usually maintain their quality on the tree for a longer time.

Relative humidity of the storage atmosphere is definitely related to the incidence of rind breakdown in citrus fruits. As mentioned previously, a low relative humidity will accelerate the rate of development of low-temperature injury of oranges, grapefruit, limes and lemons. Pitting of grapefruit has been reported to be much worse in atmospheres of low humidity (65%–75%) than in atmospheres of high humidity (85%–95%) (7). Likewise, when both oranges and grapefruit have been exposed to a dry atmosphere by storing over sulfuric acid, loss of weight, loss of firmness and increase in pitting have resulted (54).

Since the physiology of citrus fruits in storage may be dependent upon the amount of processing received, a little space will be devoted to a description of customary packing house procedures. Legally mature citrus fruits that are still green in color are first "degreened" with ethylene gas. In this process the field crates of fruit are stacked in the "coloring room" immediately after picking. A mixture of air and ethylene, with a temperature of 80° F. and a relative humidity of 90%, is circulated in the coloring room. This mixture contains one part of ethylene to about 50,000 parts of air, and the process is continued for 24 to 72 hours, depending upon the amount of green pigment (chlorophyll) present in the rind. Following the ethylene treatment the citrus fruits are dumped on a conveyor belt and run through the packing house machinery. In the first process, which consists of washing, the fruits are immersed in a soak tank containing a solution of alkaline detergent. They are then removed by a moving belt, soaped, and scrubbed by passing over rotating brushes made of palmetto fibres, while water is sprayed on them from above. Early in the season, in Florida and Texas when the fruits "degreen" with a yellow rather than an orange color, they may be given a dye treatment which enhances the final color. This is the "color-added" process and must not be confused with the ethylene or "degreening" treatment which is sometimes referred to as "coloring". "Color-added" fruit is that which has had a dye added to the rind by means of a spray or soak tank. The dye is a compound like that used in coloring butter, and all oranges so treated must be stamped "color-added". Usually the temperature of the color-added solution is maintained at from 90° to 130° F. The fruits are next dried by passing through blasts of hot air. In the commercial processing of citrus fruits it is the general custom to add a light coating of wax. This may be applied to the fruits by revolving brushes that have first picked up the wax from a solid bar, or by a hot wax vapor, or a water-wax emulsion, or the wax may be dissolved in an organic solvent and sprayed on in the form of a fine mist. The waxy coating serves both to give the fruit a polish and to retard shrinking. After receiving the wax, the fruits are polished by passing them over rotating brushes made of a soft fibre like horse hair. The next step in the packing house consists of grading, sizing and packing, either with or without wrappers. In some packing houses the fruits are given an application of borax for the purpose of reducing decay. Oranges are dipped in a saturated solution of borax either on the loading platform prior to the ethylene treatment or in the packing house prior to waxing. The temperature of the borax bath varies with the packing house and may be as high as 100° F. It is followed by a rinsing with water.

The effect of packing house processes on rind breakdown and decay of citrus fruits in storage has been studied rather extensively by Winston and Roberts (72). Working with most commercial varieties of orange they collected samples of processed and unprocessed fruits from packing houses in various parts of Florida. These authors summarized their results as follows: "The washing, color-adding, ethylene and waxing treatments given oranges in packing houses increased rind breakdown considerably. All steps appeared to contribute to the increase. . . . Decay of gassed or color-added fruit decreased with increased processing, while the amount of decay in fruit which was not colored was unaffected by processing. Juice quality, judged by total soluble solids, total acid, and vitamin C content, was not generally affected by washing, dyeing or lightly waxing the fruit. In a few instances flavor was definitely impaired". Bratley and Winston (5) found the same results with fruits after they were shipped to the New York market.

METHODS FOR PREVENTING LOW-TEMPERATURE BREAKDOWN

It is not surprising that numerous attempts have been made by research workers to develop methods for preventing or reducing physiological disorders of citrus fruits in cold storage. One of the earliest methods tried was that of holding the fruit at a higher temperature for a brief period before placing in storage or transport. This process is referred to as "quailing", "curing" or "wilting". Success with the method was first reported by Hawkins and Magness (30) in their work with grapefruit, and as a result of subsequent experiments (31, 32) recommendations were made for curing grapefruit for one to two weeks at 70° to 75° F. with a relative humidity of 65%. Similar results were subsequently reported from Texas (20). Brooks and McColloch (7) found a curing period of three to five days at 60° satisfactory, but 50° F. was not satisfactory. These authors also controlled pitting in grapefruit by heating to 100° F. for 17 to 22 hours before storing at 36° or 40° F., but this treatment increased scald and produced increased amounts of pitting when the fruit was subsequently stored at 32° F. In South Africa (62) it was found that there was an optimum period of delay for grapefruit and that prolonged delayed storage was worse than none at all. The optimum found was one to two days at 80° F. or four to six days at 65° F. Attempting to place curing on a quantitative basis, other workers in South Africa (16) reported a reduction in pitting if the grapefruit was first cured to a point where it had lost between 3.3% and 5.0% of the original weight. In Trinidad (37) it was reported that while a certain amount of wilting is desirable during the rainy season, it can be eliminated during the dry season. However, curing of grapefruit has not been adopted as a regular practice in the industry.

Curing or quailing of oranges prior to cold storage has not been found particularly advantageous (46, 56).

Attempts have been made to control low temperature injuries to citrus fruits by adding a protective cover to the fruits, either by applying a coating of oil, wax or water-wax emulsion directly to the surface of the fruits, or by enclosing the fruits individually in wrappers. Among the many types of wrappers tried are those made of tissue paper and known as apple or dry citrus wraps, or the same type of wrappers impregnated with mineral oil, paraffin or a mixture of both oil and paraffin. There are also moistureproof or semi-moistureproof wrappers such as wet-waxed paper, parchment paper, cellophane, pliofilm and aluminum foil. Control of pitting in grapefruit has been obtained by the use of a coating consisting of a mixture of mineral oil and paraffin, but this tended to increase scald and watery breakdown if the fruits were held at 32° F. (7). Beneficial results in controlling pitting of grapefruit have been obtained by wrapping with waxed paper (20), cellophane or aluminum foil (53). Some control of rind breakdown of oranges has been reported by the use of moistureproof and semi-moistureproof cellophanes, pliofilm or aluminum foil (53, 55, 70). The use of certain types of mineral oil in orange wrappers has increased rather than prevented injury.

CARBON DIOXIDE STORAGE

The use of carbon dioxide in the storage atmosphere should be mentioned here because this gas has been employed by research workers for the purpose of prolonging the storage life of citrus fruits by attempting either to prevent rind breakdown or to retard the incidence of senescence and decay. Nelson (44) first suggested that the composition of the storage atmosphere may have a direct bearing on physiological disorders in citrus fruits because he was able to produce "brown spot" (pitting) experimentally by storing oranges and grapefruit in pure nitrogen. He concluded that the disorder was caused by lack of oxygen. However, several years later Brooks and McColloch (7) decreased the amount of pitting in grapefruit by reducing the oxygen content of the atmosphere by adding carbon dioxide. Their treatment consisted of exposing the fruit to atmospheres of 20% to 45% carbon dioxide for 20 to 48 hours prior to removal to cold storage. Similar carbon dioxide treatments reduced both pitting and membranous stain in lemons (9). Stahl and Cain (54) also were able to reduce rind breakdown in citrus fruits through the use of carbon dioxide, and recommended storing these fruits in an atmosphere containing 6% of carbon dioxide and 12% of oxygen. In general, most attempts to store citrus fruits in an atmosphere of high carbon dioxide content have met with failure, the result being usually some type of injury to the rind, a deleterious effect on flavor or even an increase in fungal wastage. Results of this type have been reported by investigators in Australia, England, Palestine and Trinidad and by several in the United States (8, 14, 37, 58, 59). The periods of storage in these instances lasted from four weeks to three months, and the percentages of carbon dioxide have ranged from 5% to 81%. Although some success with relatively high percentages of carbon dioxide has been reported in England (1) the workers in the United States who have reported no injury to citrus fruits stored in atmospheres rich in carbon dioxide have either held the fruits for much shorter periods (about one week) or have employed lower concentrations of carbon dioxide (10% to 15%) (6, 8, 54, 58).

To a certain extent excessive dosages of carbon dioxide produce effects similar to those of prolonged storage. For instance, small amounts of acetaldehyde and alcohol are to be found in normal citrus fruits; but these substances increase with prolonged storage, especially at low temperatures (32° to 40° F.) and are likewise increased by treatment with high concentrations of carbon dioxide (14, 42, 43). It is possible, therefore, that the physiological disorders, previously described, are the result of auto-intoxication, caused by accumulation of the products of anaerobic respiration or of other abnormal enzymatic processes that have been induced by the environment.

ETHYLENE TREATMENT

One is tempted to give this chapter the title of "The Romance of Ethylene" because few chemical compounds have made so dramatic an entry into an industry or have continued to hold the interest of physiologists so long as has this unsaturated hydrocarbon gas called ethylene. This story begins with the beginning of the 20th century when it was customary to ship oranges to northern markets in railroad cars heated with kerosene stoves to prevent freezing. At that time it was noted that green fruits in the car assumed the characteristic orange color during transit, but this was considered to be merely a ripening process due to the heat generated by the stoves. However, in 1912 Sievers and True (50) showed that the active coloring agent was to be found in the products of incomplete combustion of the kerosene and that the heat generated was of secondary importance. Furthermore, the fruit could be "degreened" by the exhaust gases of a gasoline motor. In 1924 Denny (17) demonstrated that the active ingredient in the kerosene fumes was an unsaturated hydrocarbon gas. This suggested ethylene, a well-known gas of this type, and, accordingly, a small amount of pure ethylene mixed with air was found to color citrus fruits very rapidly. Thus began the practice of coloring or "degreening" with cylinders of ethylene gas, which is described earlier in this paper. Citrus fruits that are customarily given the ethylene treatment are the early oranges, such as Washington Navel, Parson Brown and Satsumas, as well as fruits of any variety of orange or grapefruit that fail to color properly on the tree because of having grown in the shade of dense foilage. In addition, the Valencia (late) orange usually develops a good yellow color in the winter while still immature, and then starts turning green at the stem end as it matures in the spring. These fruits are given the ethylene treatment to remove the green pigment (chlorophyll) from the rind. There is still another instance in which ethylene may be used in the processing of citrus fruits.

Lemons are picked according to size and as a result there may be green fruits among the yellow ones when they reach the packing house. The green lemons are sorted out and colored by storing at 50° to 55° F. for 30 to 60 days before marketing, but if the demand is brisk, they are subjected to forced curing or coloring with ethylene.

Degreening with ethylene is usually considered a coloring process rather than a ripening of the fruit. That is, there is no effect upon solids, acids, *etc.* that would render the fruit more palatable (73) as is the case with fruits like the banana. On the other hand there are definite physiologic changes that result from the ethylene treatment. Denny (17) found that forced curing of lemons is accompanied by greatly increased respiration and the formation of an abscission layer resulting in the loss of the stem "buttons". Ethylene treatment definitely affects the storage life of citrus fruits in that it has been reported to increase the amount of pitting and aging in oranges (72) as well as red blotch, albedo browning, and to some extent membranous stain in lemons (19), and also to accelerate decay in oranges, tangerines, grapefruit, lemons and limes (71). In accelerating decay of citrus fruits ethylene may function either as a stimulant to the biological activity of decay-producing organisms, or may produce a weakening in the cell wall structure of the host. Brooks (10) states that under some conditions ethylene stimulates the growth of the fungus *Diplodia natalensis,* one of the causal agents in stem-end decay. Other workers have found that ethylene treatment of fruits causes changes in the pectic constituents, including the middle-lamella pectin (29, 33, 41).

Interest in ethylene was revived among physiologists by the discovery that this gas is evolved by fruits and vegetables as a product of normal metabolic activity. As early as 1910 Cousins (35) reported that gaseous emanations from oranges accelerated the ripening of bananas although he was apparently unaware of what gas was being evolved. Between 1933 and 1938 a number of investigators were reporting that ethylene or a gas similar to ethylene is evolved by living plant tissue. In 1940 Biale (3) and Miller, Winston and Fisher (40) reported independently that ethylene is evolved by citrus fruits and that the evolution of this gas is more rapid in decaying fruits than in normal ones. It was also shown in both investigations that the organism that produces green mold rot in citrus fruits (*Penicillium digitatum*) is likewise capable of producing ethylene. It is beyond the scope of this paper to review the many peculiar physiologic effects that small quantities of ethylene can produce on fruits, flowers, tubers, *etc.*, in storage. Suffice it to say that the evolution of ethylene from ripening fruits in storage has often produced undesired effects on other commodities and has necessitated storing them in separate compartments. It is evident, therefore, that proper storage of citrus or other fruits requires some knowledge of living processes in plants.

SUMMARY

As citrus fruits mature there is an increase in size, weight and volume of juice, and a change from green to yellow color in the rind. There occurs a gradual increase in total soluble solids and a decrease in total acids. There occurs a slight decrease in milligrams of ascorbic acid per milliliter of juice, due to increase in juice volume, but this appears as an increase when expressed as milligrams per individual fruit. Citrus fruits are unlike some fruits in that they do not ripen after removal from the tree.

If held too long at relatively high temperatures, citrus fruits may be attacked by decay-producing organisms or they may undergo physical and chemical changes which render them unattractive or less palatable and less nutritious because of loss of solids, acids and other compounds which impart flavor and aroma to them. Retarding of these changes is accomplished by holding in cold or cool storage. Recommended temperatures for storage are: Grapefruit, 45° to 55° F. in regions where stem-end decay is not a factor, but 32° where liability to this decay may shorten the storage life; oranges, 34° to 38°; lemons, 55°–58°; and limes, 45°–48°. Thus, under proper conditions oranges may be stored for eight to ten weeks. grapefruit and limes for six to eight weeks, and lemons for one to four months without any significant losses in nutritional value. There have been reports indicating that cold storage has improved the flavor of grapefruit by causing a reduction in the quantity of the bitter principle known as naringin.

On the other hand, too long storage at certain temperatures may produce physiological disorders such as aging, brown stain or scald, pitting, watery breakdown, albedo browning, membranous stain, peteca and red blotch. Some of the factors that have been reported to predispose citrus fruits to these low-temperature injuries are a high percentage of potash in the fertilizer, a relatively high content of moisture and organic matter in the soil, the susceptibility of specific varieties, harvesting fruit after relatively high mean temperatures, storing fruit from the outside branches of the tree or fruit that is physiologically immature, processing in the packing house and low relative humidity in the storage rooms. Methods reported by research workers for reducing the amount of physiological disorders in storage consist of delayed storage, application of waxes and wrappers to the fruits, and exposure to carbon dioxide.

Rate of respiration in citrus fruit is generally a little lower than that of most fruits and vegetables. However, citrus fruits are like some of the other fruits in that after maturity they attain a respirational "climacteric", after which they go into senescence. The onset of senescence in citrus fruits has been delayed by storing them prior to the incidence of the climacteric, and the life in cold storage thus extended, although some evidence has been reported suggesting that the development of the climacteric in storage accelerates the production of physiological disorders.

Prolonged storage of citrus fruits in relatively high percentages of carbon dioxide has usually resulted in rind injury or in a deleterious effect on flavor. Investigators reporting success with carbon dioxide storage of citrus fruits have employed moderate percentages of the gas (10% to 15%). There is some evidence that a very brief treatment with high percentages of carbon dioxide reduces the amount of some of the physiological disorders subsequently developing in cold storage, but on the whole the carbon dioxide storage of citrus fruits is still in the experimental stage.

Under certain circumstances ethylene gas is employed commercially for "coloring" or degreening citrus fruits. The treatment removes the green pigment (chlorophyll) from the rinds of the fruit but does not measurably affect the solids, acids and vitamin C in the juice. However, ethylene stimulates respiration, causes the stem buttons to be released, produces certain changes in pectic substances and appears to accelerate the development of rind breakdown and decay. The increase in decay in ethylene-treated fruit may be due either to chemical disintegration of cell-wall materials, to biological stimulation of the decay-producing organisms, or to both.

In recent years citrus fruits, like other fruits, have been shown to evolve ethylene as one of the products of normal metabolism, and the evolution of this gas is more rapid in decaying fruits. The peculiar physiologic effects of ethylene on stored fruits, vegetables, flowers, *etc.*, has added to the problems of cold storage and indicates that a knowledge of the physiology of fruits and vegetables is essential to the successful storage of these products.

If a concise recommendation for storing citrus fruits is desired, it can be expressed as follows: Eliminate insofar as practicable the pre-storage conditions that have been described in this paper as undesirable. Select the temperature recommended for the particular type of fruit and attempt to maintain optimum conditions in storage. If the loss of moisture is being retarded by the use of moistureproof wrappers, inclusion of a suitable disinfectant in the wrapper is highly desirable. Make periodic inspections of the stored fruit in order to terminate the storage at the very first symptoms of development of physiological disorders.

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THE EFFECT OF MINERAL SUPPLY ON THE MINERAL CONCENTRATION AND NUTRITIONAL QUALITY OF PLANTS

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INTRODUCTION

The mineral composition of plants is a function of many factors, such as difference in soils, use of soil amendments or fertilizers, and rainfall and other climatic influences. These factors overlap in their effects or work simultaneously. One factor may influence another. Thus, the effect of climate on the plant is partly direct and partly the result of the development by climatic factors of certain soil characteristics. Differences in these factors will naturally operate to modify the mineral composition of the plant in different ways. For example, it is possible (*a*) for the mineral composition of two plants of the same variety growing in different soils to be significantly different without there being any important difference in their size or the distribution of their parts, such as leaf, stem or seed head; (*b*) for the growth (yield) of plants of the same variety to vary in different soils without any important differences in the proportions of the parts of the plants; (*c*) for two plants of the same variety growing in different soils to have quite different distributions of leaf, stem or head; and (*d*) for environmental factors to so modify the quantities of plant constituents such as protein, carbohydrate, lignin and cellulose as to influence the percentage distribution of other constituents, as by a deposition of starch with a consequent reduction in the percentage composition of the mineral elements. The properties of two soils may be such as to modify the natural flora and thus to produce plants quite different in mineral composition. The evaluation of the influence of these factors on plant composition is still proceeding, and much valuable information of fundamental importance is being obtained.

The nutritional diseases of animals which have been traced to soil characteristics may be divided into two general classes. The first, and geographically more important, are those due to a deficiency of one or more of the nutritionally important inorganic elements in the food that eventually result in bone maladies, anemias, goiter and related diseases. Diseases of the second class are the result of excesses of certain mineral elements in the soils. Of this class, selenium toxicity has been extensively studied.

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[1] The assistance of Dr. L. A. Maynard, Director, School of Nutrition, Cornell University, in evaluating the experiments involving animal feeding tests reviewed in this paper is gratefully acknowledged.

Because of modern methods of food distribution, troubles in man due to mineral deficiencies in food are rarely traceable to any specific soil condition, although such disorders as goiter are especially prevalent in areas where water and plants are deficient in iodine, indicating a deficiency of available iodine in the soil. Nevertheless, the protective foods such as milk, eggs, fruit and vegetables should be maintained at optimum nutritional levels, for a diet supplying minimum quantities of these foods may be reduced below the level required for good nutrition if the quality is inferior. Although many factors other than poor quality of food probably are responsible for nutritional troubles in both man and animals, this factor is believed to be an important one.

Fertilizer experiments have for the most part been concerned with the study of yields, and the problem of modifying the composition of the plant has been much less often the principal objective. That the composition of the plant is modified by the use of fertilizers has been known since the earliest soil fertility studies were made, and, in fact, plant composition has frequently been used as a guide for determining the nutrient requirements of the soil. For the purpose of the discussion which follows, investigations of that nature will not be considered, but an attempt will be made to assemble and organize the data and conclusions from those experiments designed primarily to improve the composition of the plant from the nutritional point of view. This field of investigation is in a state of high fluidity; ideas and objectives have not been clearly defined in many cases, and results are consequently not as conclusive as might be desired. Only a very few experiments have been reported that deal with human foods. Those concerned with animal feeds have utilized the forage crops as experimental subjects. Reviews of the effects of fertilizers and soils on crops, particularly forage crops, covering the literature up to about 1940 have been published (13, 26, 105, 116). The following review has been limited for the most part to papers appearing since that time.

EFFECT OF FERTILIZATION ON THE PHOSPHORUS CONCENTRATION IN PLANTS

The possibility of increasing the phosphorus concentration in forages has its most practical aspect, of course, where the level of phosphorus in the plant is below that required by grazing animals. The minimum level necessary for animals may vary with conditions but is believed to lie somewhere between 0.13% and 0.18% on a dry-weight basis. These low levels are adequate only for maintenance of the animal; for growth and lactation, much higher values are generally accepted as being necessary. It is of particular interest, therefore, to examine those cases in which investigators have been able to produce by fertilization forages containing adequate supplies of phosphorus where under natural conditions the levels of this element in the forage were normally very low.

Effect of phosphates. Most experiments designed to test the effect of phosphate fertilizers in increasing the phosphorus concentration of plants growing on soils deficient in this element have given positive results even though limited in magnitude. Superphosphate is, of course, the usual source of phosphates. Applications of from 100 to 500 pounds per acre are common, and higher quantities have been used in experimental work. The absolute change in phosphorus content of the plant is small, generally much less than 0.10% on a dry-weight basis, even with the heaviest applications of phosphates. Moser (86), for example, in pot tests in which he applied superphosphate at the rate of 600 pounds per acre to a Cecil sandy loam, was able to increase the phosphorus level in Austrian winter peas and lespedeza from 0.16% or 0.17% to only slightly over 0.20%. Larger quantities of phosphates did not result in any greater increase of phosphorus concentration in these forages.

Similar results have been obtained in field tests by other investigators (18, 35, 49, 60, 72, 84, 115, 120) where superphosphate alone has been applied to carpet grass, Kentucky bluegrass, lespedeza, Austrian winter peas, common vetch, wheat, potatoes and sugar beets. It is important to note that in all these studies observations have been made on single species or on mixtures that have not been altered significantly as a result of fertilization. Changes in botanical composition as a result of fertilization are commonly experienced. In South Africa (54), for example, it was found that the application of phosphates to a plot of a very deficient veld soil resulted in herbage containing 0.14% of phosphorus in one season and 0.17% in the next as compared with 0.07% in the herbage from an untreated plot. The phosphorus concentration, thus doubled, would probably supply the minimum amount within the range required in forage. However, in the original herbage *Tristochya hispida* and *Digitaria tricholaenoides* were the dominant species. After fertilization of the soil, *Digitaria tricholaenoides* increased in amount while some other species nearly disappeared. Phosphorus concentrations in the individual species are not available, but it may be presumed that a change in botanical composition might have contributed materially to the relatively large increase in phosphorus in the total herbage.

A relatively low content of phosphorus in herbage is not always a justification for expecting even a limited increase from fertilization with phosphates. In pot work with the Gila clay in New Mexico, Hinkle (56) found that the phosphorus concentration in clover was not significantly altered as a result of applications of phosphates, even though the original amount in the clover, 0.17%, was relatively low for this plant (13). In the field tests he obtained a significant increase in the phosphorus concentration in alfalfa, although the highest level he obtained was well below the average reported in the literature (13). Other investigators working with potato plants (69), alfalfa, oats and wheat hay (117) and cowpeas (7) found that applications of phosphates to the soil did not result in any significant increase of phosphorus in these plants. However, a number of investigators (7, 21, 25, 27, 48, 52, 53, 70, 75, 92, 96, 104, 117, 123, 124) have reported increases in the phosphorus concentration in forage and other crops even where the original phosphorus level was relatively high.

Application of phosphates to range lands is not always considered to be an economical procedure. In South Africa, where there is reported to be an increasing tendency to revert to stock farming in preference to grain production, it is felt that the necessary phosphorus can be supplied animals grazing phosphorus-deficient velds by supplemental feeds as rich as possible in this element. Theron (113) has accordingly studied the effect of phosphates on the phosphorus concentration in corn in this region of relatively low rainfall and dry-land cultivation practices. He reports that the annual application of 100 pounds of superphosphate per acre resulted in increases of phosphorus equivalent to 7% to 28% of that of untreated grain. Large applications up to 400 pounds did not result in any further increases in the phosphorus concentration, although crop yields were further increased. It is interesting to note, however, that recent reports from range investigations of southern Texas (16) indicate that phosphate fertilization of range lands may be more practical than feeding the mineral directly to the animal.

Effect of nitrogen. Certain of the effects of nitrogen fertilization on plant growth, such as a greater vegetative development, might well be expected to influence materially the concentration of mineral constituents of the plant. This might be particularly true when rapid and unusual vegetative growth occurs on a soil where minimum quantities of mineral elements are available. The agronomic "luxury consumption" might be considered to be zero under these conditions if the rate of uptake of mineral elements by the plant did not increase with increase in yield. However, the problem is complicated by other reactions in the soil, many of which are not clearly understood. Certain nitrogenous fertilizers undergo changes in the soil that result in increasing its acidity, and as such they might be expected to render certain mineral elements more available. Conversely, some "antagonistic" effects are also apparent, such as the depressive effect of nitrogen on calcium absorption. The net effect of nitrogen will be, therefore, a function of many soil characteristics.

Some investigators (21, 56, 108) have noted that nitrogen fertilization resulted in increased phosphorus concentration in forages, while others found either no effect (27, 49, 52, 54, 117) or a depressive effect (19, 77, 115) on phosphorus concentration. Snider (108) reported, for example, that applications of either $NaNO_3$ or $(NH_4)_2SO_4$ to Kentucky bluegrass resulted in doubling the phosphorus concentration, but even his high value, 0.16%, is below the average for this species (13). Blaser and Stokes (19) noted a reduction in the phosphorus concentration of carpet grass from 0.16% to 0.14% when 72 pounds of nitrogen per acre were applied to Bladen fine sand. Variable results were obtained on Leon soil, but the phosphorus concentration of the grass grown on that soil was well above the minimum required for grazing animals. The magnitude of increase in yield was similar for both soils.

Lorenz (72) found that the omission of nitrogen from the fertilizer mixture resulted in as low a concentration of phosphorus in the leaf blade of the potato plant as did the omission of phosphate. The highest phosphorus concentration was obtained with the highest application of nitrogen. Knowles, Watkin and Cowie (69), however, reported that nitrogen applications did not modify the phosphorus concentration of the potato plant as a whole under their conditions. Other investigators have reported only slight or no effect on phosphorus concentration of nitrogen fertilization alone on the wheat plant (53) or turnip greens (104), although in the latter case less phosphorus was found in the turnip when nitrogen was added with phosphate than when phosphate was added alone.

Effect of nitrogen with other fertilizer elements. Bledsoe and Sell (21) noted that the phosphorus content of Bermuda grass was higher when both nitrogen and phosphates without lime were applied to the soil than with either of these materials alone. Sheets *et al.* (104) found no significant difference in the phosphorus content of turnip greens as a result of applying nitrogen, phosphates and lime.

Effect of potassium fertilizers on phosphorus in the plant. There seems to be general agreement that the phosphorus content of the plant is either unchanged or lower where potassium has been supplied as a fertilizer than where it is omitted. Some investigators (19, 53, 69, 115) have noted significantly smaller concentrations of phosphorus in forages, potato plants and cereals, while others (20, 49, 54, 104) have reported no change associated with fertilizing with potassium. Leichsenring and Donelson (70) concluded from a statistical analysis of their data that potassium fertilization resulted in a lower phosphorus concentration of potato tubers, but the differences are very small and are of no practical importance. Blaser, Volk and Stokes (20) reported that the use of 1,500 pounds of limestone with 75 pounds of potassium chloride per acre had no effect on the phosphorus concentration of lespedeza.

Effect of potassium and phosphates. McClendon and Mayton (78) found under conditions in the Alabama Gulf Coast region significant increases in the phosphorus concentration in pasture herbage where potassium or limestone and potassium were added along with relatively high applications of phosphates. The level of phosphorus in the treated pastures was still low, but greatly increasing the applications of fertilizer failed to result in any further change in composition of the forage. The authors do not report the botanical compositions of the treated and untreated pastures, so that it is not certain whether this is due to a change in composition of individual species or to a change in the relative amounts of the forage species present. Sheets *et al.* (104) found no significant change in the phosphorus concentration in turnip greens as a result of applications of potassium, limestone and phosphates. Increases in the phosphorus concentration in alfalfa and sweetclover have been reported by Davis and Turk in Michigan when these crops were fertilized with potassium and phosphorus (36). It is instructive to note, however, that the level of even unfertilized forages in Michigan is much higher than in the heavily fertilized forage in Alabama. McClendon and Mayton (78) have noted this predominant effect of location within different areas in Alabama. Thus, soils and climate under these conditions may have a far greater effect on plant composition than does soil treatment. Blaser, Volk and Stokes (20) found no change in the phosphorus content of lespedeza when 450 pounds of superphosphate and 75 pounds of potassium chloride per acre were applied to the soil. Sheets *et al.* (104) found a significant decrease in the phosphorus content of turnip greens when phosphorus and potassium were applied to a number of different soils.

Effect of liming materials. It is well known that when added to acid soils, limestone represses the solubility of iron and aluminum and converts the insoluble phosphorus compounds of these elements to more soluble forms. An excess of limestone, of course, will react with the phosphates to form insoluble basic compounds. The effects of limestone, therefore, depend upon the particular conditions existing in the soil as well as on any possible interactions with other elements. The most striking effects of limestone in relation to phosphorus seem to have been on yields rather than on the phosphorus concentration in the plant. Investigators working with forages (3, 20, 24, 49, 115) in many parts of the country have not noted any change in the phosphorus concentration as a result of applying lime, although in most cases yields were increased. Some others (19, 35, 62, 78, 118, 120) noted a lower phosphorus concentration in several forage species as a result of liming. Hoover (60) reported that the phosphorus concentration of vetch in the spring was higher with liming than without but that later in the year this was not true. Fudge (50) found that applications of 400 pounds of limestone per acre resulted in a higher concentration of phosphorus in orange juice. Sheets *et al.* (104) noted no change in the phosphorus concentration in turnip greens grown on acid soils throughout the Southeast as a result of liming.

Effect of liming materials and phosphates. The reports of several investigators indicate that the combined application of limestone and superphosphate to the soil has more influence on the concentration of phosphorus in the plant than either of the materials alone. Davis and Brewer (35), for example, found that without limestone they were unable to obtain an increase in concentration of phosphorus in legumes with phosphates alone. Blaser, Volk and Stokes (20) have reported significant increases in the phosphorus concentration in lespedeza when limestone and superphosphate were applied, and others have noted increases in phosphorus in pasture herbage (46, 51, 78). Modification of botanical composition is a probable contributor to the change in the latter cases. Sheets *et al.* (104) found no significant effect of limestone and superphosphate on the phosphorus concentration in turnip greens.

Effect of nitrogen, phosphates and potassium as a complete fertilizer mixture. The foregoing reports on the effect of individual elements on phosphorus content indicate considerable variation. Studies of combinations of nitrogen, phosphates and potassium as mixed fertilizers do not offer any clarifying details with regard to these relationships. Lack of systems for sampling pastures and hays, disregard of species differences in the samples, and failure to measure normal variations are often important factors in this confusing situation. For example, Fink (48) has pointed out that a nearly threefold increase in the phosphorus content of fertilized native pasture herbage was largely due to the effect of a complete fertilizer in promoting the substitution of white clover and Kentucky bluegrass for poverty grass (*Danthonia spicata*). Other workers (27, 53, 74, 78, 101, 117, 121) also have reported obtaining much higher phosphorus contents in mixed pasture herbage as a result of fertilization with nitrogen, phosphates and potassium. As a result of application of a complete fertilizer, Vandecaveye (117) found a significant increase in the phosphorus of the first cutting of pasture herbage but not of the second cutting. He reported, however, no significant changes in the phosphorus concentration in individual species, such as alfalfa and oats and wheat cut for forage. Other investigators also have not observed any effect of complete fertilizers on the concentration of phosphorus in wheat grain and straw (53) and turnip greens (104). Tyson (115) reported a definite increase in the phosphorus concentration in Kentucky bluegrass when a mixed fertilizer was applied to one soil, although the increase was not as great as when phosphates only were added. On another soil, however, less phosphorus was found in the bluegrass from plots receiving a complete mixture than in that from the check plots. All phosphorus concentrations were well below average (13). Alben and Hammar (1), studying the effect of fertilization of Orangeburg fine sandy loam on the composition of pecan leaves, noted only slight increases of phosphorus when complete fertilizer mixtures were used.

Effect of complete fertilizer mixtures and liming materials. Smith and Albrecht (107) and Guyon (53) have reported significant increases in the phosphorus concentration in mixed hays and in individual species as a result of fertilization with a complete fertilizer and lime. These results seem to be due largely to the addition of the lime in some experiments (53), while in others (19) the application of phosphates was most effective in producing a higher phosphorus content of the plant. Sheets *et al.* (104) did not find any significant effect of this treatment on the phosphorus content of turnip greens.

Effect of other elements. Nieschlag (90) reported slight increases in the phosphorus concentration in lupine, the potato plant and the rye plant as a result of applications of magnesium compounds to the soil. Fudge (50), working with oranges, did not detect any consistent effect of even very large applications of magnesium carbonate on the phosphorus concentration in the juice. Leichsenring and Donelson (70) observed no significant change in the phosphorus concentration in the potato tuber with applications of iron sulfate to the soil. Harmer and Benne (55) found that applications of sodium chloride to muck soils resulted in higher phosphorus concentrations in celery and sugar beets only where potassium was deficient.

EFFECT OF FERTILIZATION ON THE CALCIUM CONTENT OF PLANTS

Deficiencies of calcium have seldom been reported in cattle and sheep, and Russell (100) states that "there is no evidence that grazing animals suffer from a straight acalcicosis"; but Maynard (83) points out that "generalizations regarding the adequacy of grass hay in calcium content for animal nutrition are unsafe because of the very large variations that can occur". A study of available data would indicate that most legumes will always supply sufficient calcium for animals. However, the possibility of a below-normal calcium concentration in plants and the great importance of this element in both human and animal nutrition cannot be denied. It is, therefore, of interest to examine the effects of fertilization on its concentration in the plant.

Effect of liming materials. It is recognized that liming practices tend to encourage the growth of species relatively high in calcium in both pasture and forages for hay. The growth of many of the legumes, such as white clover, soybeans, sweetclover, alfalfa and red clover, react markedly to limestone, rapidly displacing the nutritionally less desirable grasses and other plants. Since these legumes are higher in calcium than the species ordinarily constituting the native pastures, the calcium concentration in the forage as a whole is often greatly increased. While there is considerable evidence of this in the literature, there is less evidence of significant changes in the calcium concentration in individual species. Relatively small increases in calcium have been observed in carpet grass (19, 49), Austrian winter peas (*Pisum arvense*) (24, 86) and vetch (*Vicia sativa*) (24), although increased yields of these species were obtained as a result of liming. Differences in the response of species to liming have been observed by Vanderford (118). He found that the yield of and concentration of calcium in soybeans, Korean lespedeza and sweetclover were higher on limed plots but that the increase of calcium concentration was much greater in lespedeza than in the other species. Many investigators have reported no consistently higher concentration of calcium in Korean lespedeza, red top, sweetclover (3), vetch (26), corn stover, corn grain (120) or Kentucky bluegrass (3, 115) as a result of liming. In most instances, of course, yields were increased.

Calcium is of more interest in human nutrition than is phosphorus because of the greater possibility of deficiencies of this element in the diet. Consequently, the possibility of increasing the calcium concentration in plants grown for food has been considered by several investigators. Sheets *et al.* (104), in a very extensive investigation covering the soils and climatic conditions of a number of southern states, have concluded that the calcium concentration in turnip greens was not greatly affected by the calcium applied to the soil. Fudge (50), studying the composition of orange juice, reached similar conclusions, at least for normal applications of limestone. Some higher values were obtained (9.25 mg. per 100 ml. of juice as compared with 7.75 mg.) when an application of 3,200 pounds per acre of limestone was added to the soil. No consistent effect was noted on the calcium concentration in the foliage. Other workers have reported somewhat higher calcium levels in a number of vegetable crops (44), potato leaves (89) and alfalfa (62) as a result of increasing the calcium supply of the soil.

Effects of nitrogen. It has been recognized generally that application of nitrogenous manures to the soil is associated with a lower calcium concentration in the plant than is found under natural conditions. Recent findings confirm this. Ammonium sulfate as a nitrogen carrier seems to be more effective in this respect than sodium nitrate. Apparently, location, as expressed in terms of climate and soil, may modify these effects markedly. In Georgia (7), for example, the calcium concentration in cowpeas fertilized with nitrogen was lower than normal at Experiment but at Blairsville was unaffected by fertilization.' Marked reduction in the calcium concentration in carpet grass (19) and Bermuda grass (21) has been noted, particularly where high applications of nitrogen were made. Effects of nitrogen on the calcium concentration in mixed forages and pasture herbage may be associated with changes in botanical composition as well as with changes in the chemical composition of individual species. Brown and Maunsell (27) found that a lower calcium concentration in pastures fertilized with nitrogen was accompanied by a greater prevalence of bluegrass and less clover. Other investigators (12, 42, 115) have observed this effect on the calcium concentration in pastures, but botanical analyses have not always been reported.

Knowles, Watkin and Cowie (69) and Lorenz (72) found that applications of nitrogen resulted in lower concentrations of calcium in the leaves of potato plants. Sheets *et al.* (104) reported that in all possible fertilizer combinations of nitrogen, potassium, phosphorus and calcium, nitrogen produced the most marked effect on calcium in turnip greens—that is, significantly lower values for calcium were obtained in 24 out of 30 experiments in which nitrogen was applied as a fertilizer. Guyon (53) reported little effect of nitrogen on the calcium concentration in wheat grain or straw. Vandecaveye (117) noted little effect of nitrogen fertilization on the calcium concentration in several forages in Washington. Bledsoe and Sell (21) found that nitrogen and phosphate fertilization resulted in lower concentrations of calcium in forages. Even the addition of lime failed to establish a calcium content per unit weight equal to that in forages from unfertilized soils in Georgia.

Effect of phosphates. In field practice phosphorus is ordinarily applied to the soil as a calcium phosphate along with calcium sulfate (superphosphate). Consequently, the effect of phosphorus alone on the calcium content of plants can seldom be measured. In common with the results on the influence of limestone and other calcium compounds, many investigators have reported very slight or no change in the concentration of calcium in plants as a result of the use of superphosphate as a fertilizer (21, 53, 60, 70, 78, 84, 86, 92, 107, 117, 120). In a few instances, superphosphate has been associated with higher percentages of calcium in carpet grass on certain soils but not on others (19), while in the same region, Florida, it was noted that no change occurred as a result of the use of superphosphate if liming were ample (20). Significant increases in the calcium concentration in pastures without a change in plant population have been reported (115, 123) as a result of fertilization with superphosphate. Similar results have been found for the potato leaf (72). Sheets *et al.* (104) reported significantly less calcium concentration in turnip greens fertilized with superphosphate as compared with no superphosphate. Hirst and Greaves (57) have reported that the calcium concentration in sugar-beet leaves grown on soil fertilized with calcium sulfate was lower than in those from untreated plants. Much more information concerning the behavior of gypsum in superphosphate is needed to clarify the effects of this calcium carrier.

Effect of potassium. Investigators using a wide variety of species and soils are almost unanimous in their conclusions that applications of potassium result in a repressed absorption of calcium by the plant accompanied by a lower concentration of the element in most instances (23, 40, 45, 53, 57, 69, 72, 82, 102, 115, 120), although it has been observed that where no increase in yield occurred, the calcium concentration in some forage species has not been affected materially (49, 115). Others (19, 20, 104, 117) also have observed no effect of potassium on calcium concentration, but relationship to yield is not always apparent. Hunter (61) reported a marked increase in the concentration of calcium in alfalfa as a result of increasing calcium supply and decreasing potassium supply in a Dutchess loam soil.

Effect of nitrogen, phosphates and potassium as a complete fertilizer mixture. The marked influence of soil and climate on the effects of fertilizer treatment is again demonstrated in the work in Georgia, where at Experiment (7) the calcium concentration in cowpeas was lower with application of complete fertilizers, while at Blairsville no difference between fertilized and unfertilized soils was noted. Lucas, Scarseth and Sieling (73) found that heavy applications of complete fertilizer mixtures resulted in decreases in calcium concentration in mixed hay, alsike clover and red clover. The calcium concentration in corn, wheat and soybean plants was not affected. Most reports (42, 53, 70, 78, 104, 117)are negative with respect to any marked effect of applications of complete fertilizers on the calcium concentration in plants, although yields were commonly increased as a result of their use.

Effect of complete fertilizers and liming materials. The effect of climate in modifying the influence of soil treatment is suggested by the work of Blaser and Stokes (18), who reported no significant change in the calcium concentration in carpet grass (*Axonopus affinis* Chase) in 1937 as a result of applications of complete fertilizer and limestone, although very significant increases were obtained in 1939 and 1940. Yields also were very much greater in the latter years. The authors do not attempt to explain this difference except to ascribe it to climatic conditions. Other investigators (73, 115) have noted increases in calcium concentration in some forages when both limestone and complete fertilizer were used, but not all crops responded equally. No response was noted in wheat grain or straw (73), timothy (60, 107) or turnip greens (104).

Effect of other elements on the calcium content of plants. There is a general agreement in recent work (28, 44, 45, 50, 90, 122) that applications of magnesium to the soil appear to reduce the calcium concentration in plants. The differences are often small and in most cases may be of no practical importance except where the calcium level is normally low. For example, Willard and Smith (122) reported from 13% to 32% less calcium in various species grown on magnesium-fertilized plots, but only in timothy did the calcium concentration approach a level believed to be limiting with respect to nutritional value. Sulfur may have the effect of increasing the calcium absorption by the plant, according to the findings of Chapman and Brown (29) in experiments with orange trees.

EFFECT OF MICRONUTRIENTS ON THE MINERAL COMPOSITION OF PLANTS

Increased boron concentration in a number of plants has been demonstrated in several instances (33, 67, 88) where boron was added to the soil. The effect of boron supply on the utilization of other elements by the plant seems to be more variable. Boron had no effect on the calcium or phosphorus concentration in a number of forages in the experiments of Nowosad (91) and Cook and Millar (33). Jones and Scarseth (67) also reported little effect of boron on calcium concentration except at very high levels of boron supply, where yields were reduced. Under these conditions, the calcium concentration in alfalfa and oats was reduced. Applications of boron to the soil have been shown to have reduced markedly the iron concentration in sugar-beet roots and spinach leaves (71). Additions of limestone may reduce the concentration of boron in plants (67, 88), although applications of boron with limestone generally restored boron concentration to its original level in these experiments.

Teakle (114) has reported that application of from two and one-half to ten pounds of bluestone per acre to pasture land will raise the copper content of the herbage from 3 to 7 or 12 p.p.m. Johnson (66) has reported that sulfur had no effect on the copper concentration in alfalfa growing on a sulfur-deficient soil. MacIntire (80) found no increase of fluorine concentration in Sudan grass from applications of calcium fluoride to soils in pots.

There is little evidence that addition of iron compounds to the soil has resulted in increased iron concentration in plants. At least the differences that have been reported are variable and of little practical importance (70). Additional recent evidence that liming the soil would reduce the iron (93) and manganese (14, 27, 93, 110) content of plants under certain conditions has been obtained.

No effect of gypsum on the iron concentration in alfalfa (66) or turnip greens (110) has been observed. In an extensive factorial experiment, designed to test the effect of all combinations of nitrogen, phosphates, potassium and calcium on the concentration of iron in turnip greens (110), it was found that a significantly lower iron concentration was associated with nitrogen fertilization. Brown and Maunsell (27) found a lower iron content in pasture herbage where phosphates and potassium had been applied. When limestone was included in this treatment, no further effect on iron was noted.

The effects of liming materials on the micronutrients in forage have continued to receive attention. Albrecht and Smith (4) in pot tests found that mixing the limestone throughout the soil resulted in less absorption of manganese than occurred when the limestone was incorporated in the surface two or three inches only. Additions of sulfur (93) and ammonium sulfate (109) have been reported to increase manganese absorption. Johnson (66), however, did not observe any change in the manganese concentration in alfalfa growing on a sulfur-deficient soil fertilized with gypsum. It has been noted (4) that applications of phosphates were associated with increased manganese concentration in the plant.

Ferguson, Lewis and Watson (47) have found that use of limestone or other basic materials on pastures favors absorption of molybdenum by the herbage, whereas ammonium sulfate and other acidic materials have the opposite effect. Millikan (84) found little effect from ten pounds of zinc per acre on the calcium, phosphorus, zinc or manganese concentration in wheat plants. There was a slightly greater zinc concentration, however, where superphosphate had been applied. Harmer and Benne (55) could find little effect of sodium chloride on the calcium concentration in plants except with very high applications that depressed the absorption of calcium.

Additional work has confirmed the fact that the cobalt level of pasture herbage can be successfully increased by use of cobalt compounds (9, 111), of cobaltized superphosphate (8, 22, 68) or of limestones high in cobalt (38, 99). In areas where soils are low in cobalt, application of nitrogen to increase yields may reduce the concentration of cobalt in the plant (63), although where the supply is adequate this will not always occur (14).

INVESTIGATIONS WITH NUTRIENT SOLUTIONS

A limited amount of recent work in solution cultures has contributed materially to our knowledge of how mineral supply acts in modifying the mineral concentration in plants. In addition to observations that the quantity of calcium and phosphorus found in the plant are proportional to the supply in the nutrient solution, some effort has been made to study interactions. The contributions in the latter field have included studies with both major and minor elements.

Phosphorus. It has been observed that phosphorus concentration in the plant is depressed by a deficiency of magnesium in the culture solution (15). Phosphorus concentration seems to be inversely proportional to the supply of potassium in the nutrient solution in some cases (11, 34, 98), although Bartholomew (11) found that while this was true in the leaves of tomato, the reverse was true for the stems. McCalla and Woodford (77) observed that limiting nitrogen in the culture solution resulted in increased phosphorus concentration in the plant. It also appears that a high nitrogen supply may be responsible for depressed phosphorus content per unit of dry matter (34, 106). Scott (103) did not note any change in phosphorus in the plant where the ratio of potassium to sodium, and of potassium to magnesium, were increased in the nutrient solution throughout a considerable range. Richards and Sheng-Han (98) noted an increase in the phosphorus concentration in barley with increase of the sodium supply in the nutrient solution. Dickman and DeTurk (37) found that young corn plants grown in sub-irrigated gravel cultures and supplied only with phosphate rock as a source of phosphorus were limited in growth and phosphorus content as compared with those supplied a soluble source of phosphorus. In general, nutrient culture work is not a satisfactory guide for soil phosphorus studies because of the great differences in the level of phosphorus supply and the character of the phosphorus compounds in the soil as contrasted with the conditions in nutrient solutions.

The hydrogen ion concentration of the solution is of importance in the calcium-phosphorus interaction, according to Moser (87). At a low pH, an increase in calcium supply did not result in any change in the phosphorus concentration in soybeans, although at a pH of 6.0 to 6.5 there was an increase in phosphorus. There is some evidence of species differences, however, for the phosphorus concentration in lespedeza increased with increase of calcium supply at all pH levels, while no changes occurred in the phosphorus content of sorghum. Chapman and Brown (30) found no differences in the phosphorus concentration in orange leaves from seedlings grown in nutrient solutions with either high or low calcium supply.

There are some indications that high iron supply in nutrient solutions may be associated with a decreased concentration of phosphorus in plants (106), but the differences are of little practical importance. Bartholomew (11) has pointed out that an abundant nitrogen supply actually increased the concentration of iron in plants in his experiments.

Calcium. Sideris, Young and Krauss (106) and Jacobson and Swanback (64) found that the concentration of calcium in plants was greater in the presence of nitrate ions than of ammonium ions in the nutrient solution. Calcium concentration in the plant tends to be inversely proportional to the supply of potassium in the nutrient solution (10, 15, 30, 112), although Scott (103) found no change in the calcium concentration in *Chlorella pyrenoidosa* with an increase in the ratio of potassium to sodium in his solution and a marked increase of calcium as he increased the ratio of potassium to magnesium over a wide range. It is generally agreed (10, 15, 71, 87, 112) that the calcium concentration in plants will increase with increase in the calcium supply of nutrient solutions. In other experiments (106), iron had no effect on the calcium concentration in the test plant. Barbier (10) reported that magnesium depressed the calcium concentration in a number of plants, but others (15), using the tomato plant, did not confirm this.

Studies with soybean (85, 119) and tobacco (112) have demonstrated that the calcium concentration in these plants grown in nutrient solution is roughly proportional to the boron supply when sub-optimal supplies of boron are present. When super-optimal supplies are present, this relationship becomes an inverse rather than a direct one (85). In general, when boron supply was optimum for growth, the calcium content of the leaf blades of the soybean was at a maximum. In the corn plant, however, boron supply did not greatly influence calcium concentration, although both boron and calcium concentrations in the plant were directly related to the supply of each element, respectively, in the nutrient solution (81).

Minarik and Shive (85) point out that the inconsistent results so far obtained as to the effect of boron supply on calcium metabolism suggest that if boron actually does influence calcium metabolism, its effect varies with the species of plant investigated and with the conditions under which the experimental procedure is carried out. Lorenz (71) found that an increase in boron supply in the nutrient solution resulted in increased growth of garden beets and increased absorption of calcium, but no increase in the content of calcium per unit of dry weight. He believes that boron may be more closely associated with the utilization than with the absorption of calcium. Reeve and Shive (97) conclude that calcium accumulation in the tissues of tomato plants is largely determined by calcium supply and appears to be independent of boron. Similar conclusions have been reported with the cotton plant (59).

Micronutrient elements. A high level of calcium supply has been associated with reduction in concentration of manganese and iron (112) in tobacco plants grown in nutrient solutions. Most investigators using nutrient solutions have shown that the concentration in the plants of micronutrient elements iron, molybdenum, boron, manganese, zinc and copper is dependent upon the level of supply available in the culture solution (6, 39, 59, 76, 81, 85, 106). Reeve and Shive (97) found that for any given boron supply in the substrate there is a progressive increase in the concentration of boron in the plant as the potassium supply increases. This was especially pronounced at high levels of boron supply. Chapman and Brown (30) found that when potassium was omitted from the nutrient solutions, peach tree leaves absorbed excessive quantities of boron. Reeve and Shive did not work with these low levels of potassium. Sideris, Young and Krauss (106) reported an inverse relationship between iron supply and manganese concentration in *Ananas comosus*. Chapman (31) found that excessive zinc supply in sand cultures resulted in a reduced iron concentration in lemon plants.

Others (94) have studied the effects of boron supply, ranging from deficient to toxic concentrations, on the chemical composition of tomato leaflets. The concentration of 14 elements was determined in the plant, and it was found that there were significant and large differences, depending upon the boron supply. The concentration of some elements was altered as much as several hundred per cent.

EFFECT OF FERTILIZATION ON THE QUALITY OF FORAGES AS MEASURED IN TERMS OF ANIMAL GROWTH AND HEALTH

It is not uncommon to measure the returns from pasture fertilization in terms of increase in production of animal products per acre. Increases in yield and the introduction of more nutritious forage species have been shown to improve the grazing value of land in terms of weight gains of animals or increase in days of grazing (5, 16, 17, 27, 41, 43, 51, 54). To test the effect of fertilization on the nutritive value of a single forage species is a more difficult matter and is not commonly done. Fundamentally, however, this is a question of considerable interest and importance.

Albrecht and his co-workers (2, 58, 79, 107) have attempted to measure improvement in the biological factors in forage plants resulting from the use of fertilizers, particularly calcium and phosphates. Their general approach has been to use animal growth as an index of the changes in crop quality in response to soil treatment. This is based on the justifiable assumption that the animal might respond to changes in the composition of the forage that are not detectable by ordinary chemical analyses. They found, for example, that lespedeza grown on soil treated with both phosphate and lime contained about 17% more protein than that from untreated soil. They conclude that it is not unreasonable to suppose that organic compounds other than proteins also may be influenced favorably.

In their experiment, carried on in the fall of 1939 (2), they report a 50% greater gain in growth of lambs fed lespedeza from soils treated with limestone and superphosphate as compared with that from untreated soils. Unfortunately, their experimental hay crop was partially destroyed by rain after harvest, and the portion recovered was supplemented by other hay grown under uncontrolled conditions. It is necessary, therefore, to consider very critically any conclusions in regard to the effect of soil treatment on crop quality in this experiment. This was recognized by these investigators, and the experiment was repeated the following year when conditions of harvest were more favorable. In this experiment, it was found that sheep fed lespedeza hay from limed soils gained 0.1644 pound per day as compared with 0.1408 pound for those on lespedeza from unlimed soils. The composition of the two hays was not signficantly different with respect to the constituents determined—nitrogen, calcium and phosphorus. The authors conclude, therefore, that the animals responded to differences in forages not detected by chemical analyses.

A careful scrutiny of the data, however, reveals the following: (*a*) apparently no account was taken of possible variations in botanical composition; (*b*) no report was made as to the proportion of leaves to stems in the two hays; and (*c*) no attempts were made to determine the digestibility of the two hays. That these factors were probably of considerable importance is shown by the fact that about 50% of the hay from unlimed soils and 46% of the treated hay was refused by the animals. This is an unusually large waste in an experiment of this nature. Where wastage is of this order, it is manifestly impossible for one to conclude that the hay actually eaten is representative of the soil treatment. Furthermore, the difference in gains of weight between the two sets of sheep is very small (0.1408 lb. and 0.1644 lb. per head per day), and data are not presented to show that this small difference is significant. Thus, normal variation might well account for these differences, and the greater gains might just as well have been made by either group of the sheep if both had been fed identical hays. In view of these discrepancies, the authors' conclusion that although "there were no differences in the concentrations of calcium or phosphorus in these hays, yet in terms of animal nutrition the presence of lime must have altered the physiology of the lespedeza plants during growth" must be accepted with a great deal of caution. In a later experiment, others (79) did observe that there was an appreciable difference in the botanical composition of their treated and untreated hays, much more grass appearing in the untreated materials.

The variability in biological measurements of the feeding value of forages is demonstrated in the work of Albrecht and Smith (107). In most of their experiments, they use only three animals, whereas a minimum of six to eight is usually considered necessary for this type of work. It is interesting to note, however, that though their measurements sometimes indicate a slight difference in favor of crops from soils treated with superphosphate or superphosphate and limestone, at other times they indicate that the crops from untreated soils appear to be the best. The authors, of course, seek to explain these anomalies, but the critical reader needs a nearer complete presentation of data to gain some appreciation of variability. The presentation of averages, particularly when the differences are not great and the results do not consistently follow some reasonable trend, is not justified in any biological work. The approach of these investigators is a step forward, however, and the concept of measuring the overall nutritive value of forages as a measure of fertilizer efficiency deserves more attention.

DISCUSSION

It is apparent that studies of the effect of fertilization on the mineral composition of plants have not yet produced sufficient data obtained under a sufficient variety of conditions to permit many generalizations. Thus, most of the recognized factors, such as soils, climate and plant species, that modify the influence of fertilizers, have been studied in little detail. Plant composition is the end result of a number of physiological processes, many of which are controlled by environmental factors whose influences are little understood. Even in carefully controlled nutrient solution culture work, the relationships of cause and effect are not always clear. Under such experimental conditions, however, the consistency of some results indicates a correlation of certain combinations of nutrient supply with certain net effects in the plant. Thus it seems to be generally true that the calcium concentration in the plant is negatively correlated with potassium supply in the nutrient solution. It is interesting to note that there is also a remarkable unanimity of agreement on this relationship as a result of soil experiments.

Although a direct translation of the results obtained in this way to the conditions in any soil is still not advisable in all respects, any interpretation of the composition of plants in terms of a soil's characteristics and the fertilizer applied can be made only by taking into consideration the relationships demonstrated in culture solutions. Peech and Bradfield (95) point out, for example, in their analysis of the calcium-potassium interaction in soils, that plants absorb the greater part of their nutrient cations from the soil solution. The interchange of ions on the solid phases of the soil system renders the whole relationship more complex, however, than is true of the simple solution culture. Thus, Jenny and Ayers (65) have shown that the exchangeability of absorbed potassium is proportional to its percentage saturation of the exchange capacity of the colloid. Furthermore, this relationship is greatly affected by the nature of the complementary ion. This means that addition of any salt to the soil that would displace one of the absorbed ions from the colloid would upset the existing ratio of ions in the soil solution and consequently influence the uptake of one or more ions by the plant.

The variability of plant composition with fertilization on soils of different kinds and origins is therefore not surprising, for the effects of the soil colloidal material and of the soil solution are responsible only in part for these differences in response. Differences in climate, native vegetation and soil parent materials also contribute to these effects. Thus, the Podzolic and Lateritic soils are well-leached acid soils generally quite low in bases and phosphorus, while the Chernozem and Chestnut soils, which are not leached, are high in base constituents and are nearly neutral in reaction. Within

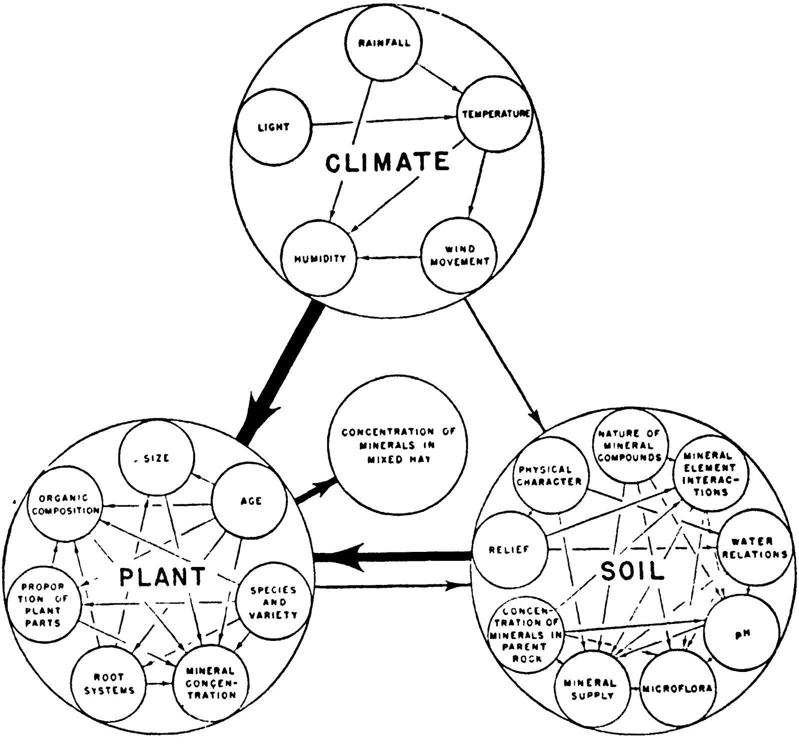

FIG. 1. Factors affecting the mineral composition of a mixed hay. The width of the arrows indicates qualitatively the effects of climate and soil.

any of these soil groups, moreover, there will be variations in fertility and reaction because of differences particularly in parent material and relief. For example, in the Chernozem soils deficiencies in potassium and phosphorus may exist where the parent materials are very low in these elements. In the rolling and hilly sections of the Kentucky and Tennessee bluegrass regions, erosion removes the acid and leached materials of the soil as rapidly as they are formed, the soil that remains is young and relatively immature, and calcareous material often high in phosphorus lies within the reach of the plant roots.

In Figure 1 an attempt has been made to summarize graphically the known factors that would affect the mineral composition of a mixed hay. These are divided in three parts: (*a*) The discrete elements of climate, (*b*) the chemical, physical and topographic characteristics of soils, and (*c*) the character and development of plants. It is evident that within any one group one must deal with a highly complex and relatively little understood system. Thus, the water relationships in soils are known to affect profoundly the mineral supply of the soil, but the magnitude and nature of these effects are probably greatly modified by other factors, *e.g.*, the physical character of the soil and the nature of the mineral compounds present. Likewise, in studies of plant composition, the factor most commonly ignored is the part played by differences in the proportion of plant parts such as the leaves and stems.

In this scheme (Figure 1) it is intended to stress both the direct and indirect effects of climate on the mineral composition of the plant. The direct effect is probably greater than the indirect effect that is derived by the plant from the soil. It should also be noted that the plant plays its part in the development of the soil and that this in turn has its influence on plant composition. When one considers, therefore, the complexity of the biological system under which we produce our food crops, the variable results obtained by the superimposition of a fertilizer will be recognized as natural. The problem is one that requires detailed study of each of these factors and of its effect on the others, as well as determinations of the overall or net effects as found in each soil as a unit.

Two deficiencies of fundamental importance are generally characteristic of much of the work reported in this review. First, only a few workers have attempted to design experiments that will measure variability. It is well known that when a single experiment is replicated several times, different results are usually obtained. This is because a number of factors that influence results cannot be controlled. It is almost impossible to repeat any type of biological experiment under practically the same conditions and obtain exactly the same result. Plants growing side by side in the greenhouse and under the most exacting control may not be exactly alike in composition. The necessity for experiments designed to measure the effect of uncontrolled conditions must be recognized before any significant advances can be made in a study of factors affecting plant composition.

Secondly, while the objective of most fertilizer experiments with forage must be the over-all improvement of pasture or hay, the fundamental importance of the effect on single species should not be ignored. Collander (32) has pointed out that single plant species are constantly found to be relatively rich in certain cations, and other species are rich in other cations. Thus, it can be demonstrated repeatedly that applications of lime or nitrogen will have profound effects on the botanical composition of pasture herbage and consequently on the chemical composition of the herbage without regard to that of the single species.

The need for standardizing the nutritive value of plants in terms of animal health is obvious. Certainly any limited number of laboratory determinations is inadequate, and our information does not permit an evaluation of all known factors to be made by this method. Therefore, properly planned animal experiments with crops of known history, accompanied by detailed chemical study of the crops with respect to the nutrients required by animals, should prove very useful in evaluating the effects of soils and fertilization. In undertaking such experiments, however, it must be realized that animal performance is subject to as many variables as is soil fertility and that these variables must be taken into account in planning the experiment and in interpreting the results. There are differences in the nutritive value of rations which are not measurable in terms of growth performance but which require refined physical, biochemical and histological techniques. Two crops may vary widely in the content of a given nutrient and yet the differences may not be evident unless the crops are fed at restricted levels of intake in rations otherwise adequate in all respects. Without control of the variables involved in animal experimentation, differences in results may be incorrectly attributed to certain known soil and plant differences. For example, marked changes in growth performance may result merely from differences in palatability or from the general unsuitability of the rations for the species in question.

Animal experiments are essential for the evaluation of certain nutritive differences such as those based upon variation in the amino acid make-up of proteins and upon vitamin differences not susceptible to determination by chemical or micro-biological methods. They are useful in searching for undiscovered differences in nutritive value and undiscovered nutritive needs. They also must be the final test of the differences in nutritive value which are measured by chemical or other means.

SUMMARY

From studies of the effect of soil fertilization on the nutritive value and mineral composition of plants, it is possible to make only a few generalized conclusions at this time:

1. The concentration of phosphorus in most species utilized for forage can be increased slightly by use of phosphate fertilizers, particularly where the original phosphorus concentration in the plant is unusually low. There seems to be evidence, however, that the phosphorus concentration in any species may be relatively resistant to change. There is considerable evidence to indicate that the response of phosphorus concentration in the plant to application of phosphates to the soil is dependent to a large degree on the nature of the soil.
2. The effects of nitrogen applied to the soil on the concentration of phosphorus in the plant are inconclusive whether the nitrogen is applied alone or with other fertilizer elements.
3. Any effects of potassium alone or with other elements on the concentration of phosphorus in plants seem to be variable and of little practical significance.
4. Liming the soil seems to depress the concentration of phosphorus in plants under some conditions, while no effect is evident under others. This is probably a soil factor. Liming accompanied by application of phosphates or complete fertilizer mixtures has been found effective under some conditions in increasing the phosphorus concentration in plants.

5. Of several other elements studied, only applications of magnesium to the soil seems to be associated with an increase in the concentration of phosphorus in the plant.

6. In general, liming the soil has resulted in a higher concentration of calcium in the plant, although it is evident that plant species and soil conditions will act to modify this factor measurably.

7. The effect of nitrogen in reducing the percentage of calcium in plants has been confirmed under a number of widely variable soil conditions and in many plant species.

8. Application of calcium as superphosphate has not ordinarily been associated with any change in the calcium concentration in the plant.

9. A reduction in the concentration of calcium in plants has generally followed potash fertilization. No positive correlations of calcium in plants with potassium supply have been reported.

10. There is general agreement that applications of magnesium to the soil result in a lower concentration of calcium in plants, although the differences are seldom of practical importance except where the calcium level is already low.

11. The number of soil experiments in which the micronutrients have been studied are too few to permit any generalizations as to their influence on the general composition of the plant. The data available suggest some important interactions, and these are supported by the work in solution cultures. It is generally true that applications of boron, cobalt, copper and manganese result in greatly increased absorption of these elements by the plant.

12. There is some evidence that liming practices and intensive fertilization under conditions of a limited supply of micronutrients such as boron, manganese, iron and cobalt may further reduce the amount of these elements in the plant.

13. The desirability of correlating the results of animal feeding experiments with plant composition studies is emphasized. Sound and precise animal feeding experiments designed to test the effect of fertilization on the nutritional value of forages are indicated as one of the approaches that will reveal needed information in this field of research.

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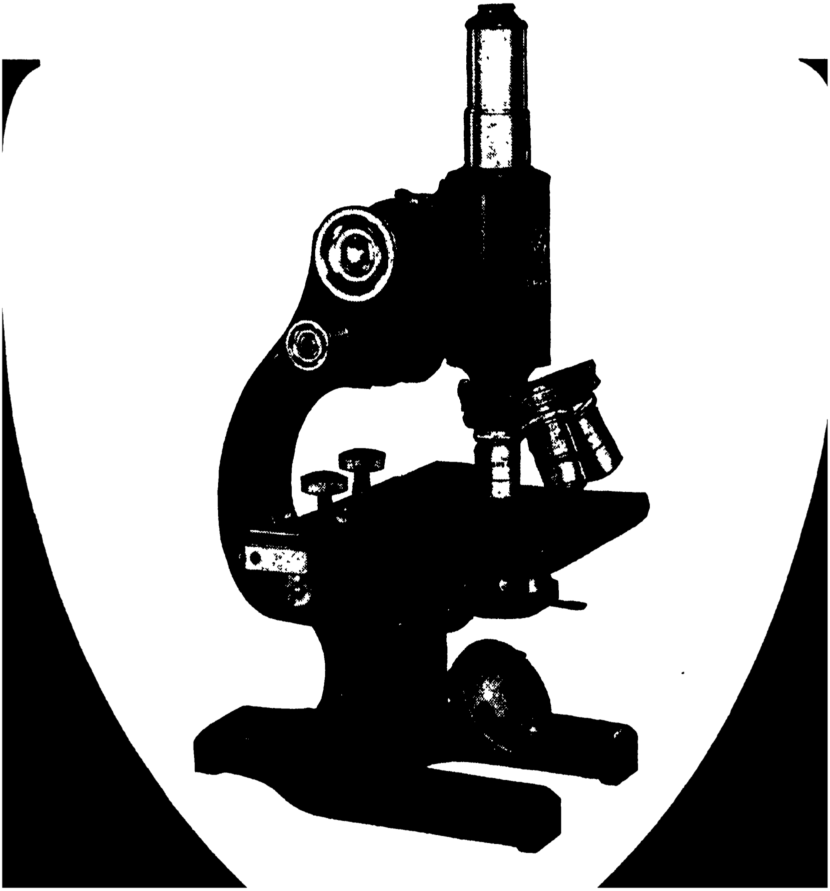

THE BOTANICAL REVIEW

Vol. XII October, 1946 No. 8

FATUOID, SPELTOID AND RELATED MUTATIONS OF OATS AND WHEAT

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A. INTRODUCTION

Though the most commonly cultivated oat and wheat species are probably of considerable age (115, 142, 156) and both are commonly self-fertilized and therefore tend to exist as "pure lines", they continue to produce occasional off-type progeny. The most striking of these resemble wild or other related species. Darwin (36) and his contemporaries were interested in these off-types and "intermediate" forms for their bearing on the variation and evolution of cultivated plants. So also, towards the end of the 19th century, were many systematists. In the early years of this century geneticists were interested in them for their bearing on the problem of mutation and the origin of variations. When, in 1918, it was discovered that cultivated oats and wheat are polyploids with three times as many chromosomes as occur in the simplest species of *Avena* and *Triticum*, it became evident that an analysis of the nature and origin of the off-types would depend upon and contribute to an understanding of the special cytogenetics of naturally occurring polyploid species. Such an analysis is, of course, of practical value to the plant breeder as well as being of evolutionary and general cytogenetic interest. Further, the results have economic significance for seed growers and agriculturists in general, because the off-types are at best undesirable impurities in fields or in seed grain, and one of them, the "false wild oat" or Fatuoid, is often confused with real wild oats, which are a "noxious weed"[1].

Besides those off-type oats and wheat that resemble wild or other related species there occur, of course, many others such as colour and chlorophyll mutations, and dwarfs, but these will be considered only incidentally in the present review.

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[1] The reader interested in the mutational or cytogenetic aspects of the problem but not in the historical, evolutionary or purely agricultural ones is referred to Huskins (77).

Oats

Buckman (16) reported that "by cultivation and selection . . . *Avena fatua* has been made to assume the form of different varieties of cereal or cultivated oats", and further, that "the cultivated by degenerating may relapse into the wild state". In the light of present knowledge it seems probable that Buckman started his "improvement" work with natural hybrids between cultivated and wild oats, and, from his description, at least one of the "degenerating" forms appears to have been of the type which later became known as the false wild oat (33) or *fatuoid* (130). These resemble *A. fatua* in those awn and glume characters which are its chief diagnostic features, namely, that each grain of the spikelet has a twisted geniculate awn and an oval disarticulation surface, surrounded by dense pubescence (Fig. 1).

But in other respects, such as colour and shape of panicle, they are similar to the particular cultivated variety in which they occur. Almost invariably the fatuoids are segregates from heterozygous fatuoids, these latter really being the forms whose origin from normal cultivated oats has to be explained. The heterozygous fatuoids are more or less intermediate between the normal and the fatuoid in most of the characteristics which distinguish the latter forms. The heterozygous fatuoids have a twisted geniculate awn on only the primary grain of each spikelet. Secondary and tertiary grains of the spikelets are characteristically awnless. The base of the primary grains is nearer that of *A. sativa* than of *A. fatua,* and it is indistinguishable from the former on the upper grains. The fatuoids are readily recognized either in fields or in threshed oats, but heterozygous fatuoids often pass unnoticed. Fatuoids are, generally speaking, true breeding forms and hence are often called "homozygous fatuoids".

Forms intermediate between *A. fatua* L. and the cultivated *A. sativa* L. received much attention from systematists shortly after Buckman's time. A careful analysis of the differences between *A. fatua* and *A. sativa,* and of their relationship, was made by Haussknecht (62) in 1885. He found that these two species are linked by a series of "transition forms" in which the "wild" characteristics of *A. fatua* are gradually lost: the oval disarticulation surface or "sucker mouth" of the grains, which causes them to shed when ripe, is solidified step by step until disarticulation is possible only by

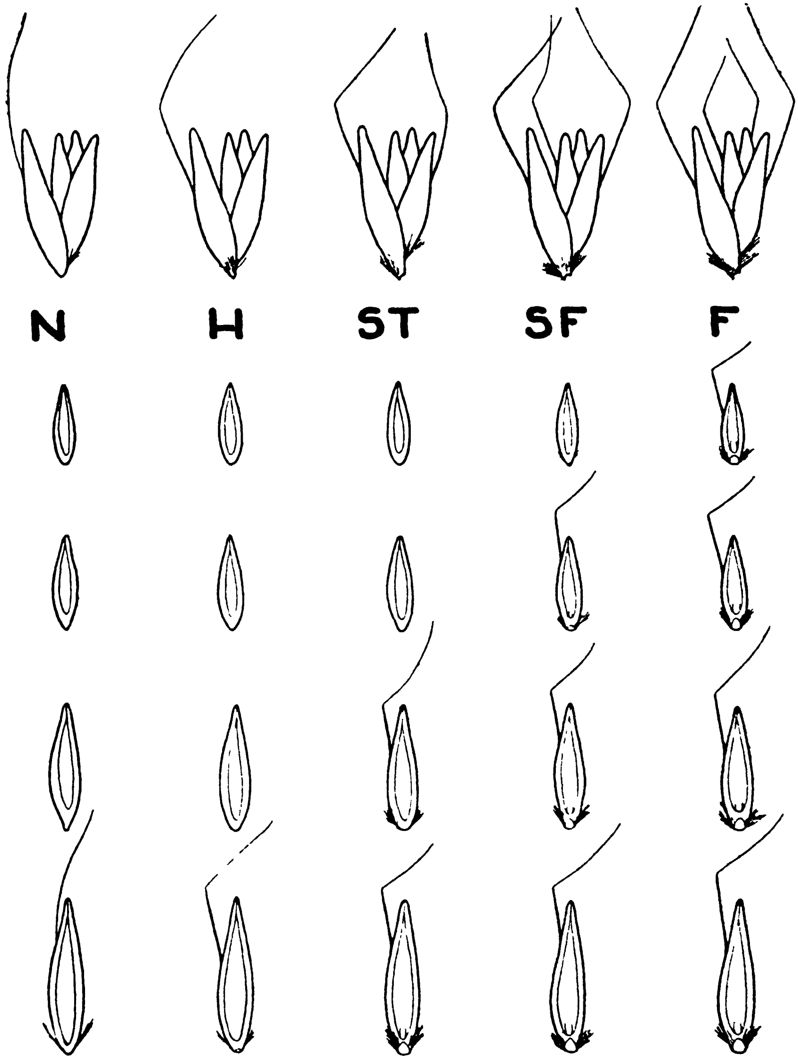

FIG. 1. Diagrammatic representation of the glume (hairiness and articulation) and awn characters differentiating normal (N), heterozygous mutant (H), steriloid (ST), subfatuoid (SF) and fatuoid (F) oat spikelets.

fracture, the pubescence on the back of the glumes disappears, and the awn either disappears or is reduced to a thin, weak structure. Haussknecht believed that in his analysis of this series of transition forms he had shown the gradual evolution of *A. sativa* from *A. fatua.* He therefore rejected the then prevalent classification of Durieu de Maisonneuve and Cosson (27) in which the Euavenae, on the basis of disarticulation character, were divided into the Sections Agrestes and Sativae; this assigned *A. fatua* and *A. sativa* to widely different taxonomic units, while within each Section were species only remotely related—some, as we now know, with widely different chromosome constitutions (see Section D). One intermediate form which Haussknecht designated *A. fatua transiens* was evidently a segregating hybrid form, since he reports that by selection he obtained typical *A. sativa* from it within four years. At first he had considered it a hybrid between *A. fatua* and *A. sativa* but, in accordance with contemporary standards, abandoned this opinion when he found it fully fertile. His description of it reads much like that of a heterozygous fatuoid, and Zade (213), neglecting the important point that it had a few hairs on the glumes, regarded it as such (see Section B).

Haussknecht's work was disregarded by many taxonomists, and intermediate forms, instead of being considered as indicating the evolutionary trend, were all assumed to be hybrids of *A. sativa* × *A. fatua* (10, 98). Thellung (175), who evolved the modern polyphyletic system now generally accepted for the Euavenae, vindicated Haussknecht's general views, and about the same time Trabut (182–184) gave evidence that the Mediterranean cultivated oat, *A. byzantina* Koch (*A. sterilis culta* Marquand), has been derived by a series of transition forms from *A. sterilis* L., not from *A. fatua.* Some of Trabut's intermediate forms had been described earlier by Haussknecht who, though he did not have the Mediterranean form, said that cultivated oats could be derived from both *A. sterilis* and *A. fatua.* The evolution of *Avena* and the interrelations of its species is still an unsettled problem, and it should be noted that the specific names used here are not necessarily those most acceptable to modern taxonomists, but those currently in most common use by agriculturists.

The intermediate or transition forms remained of purely evolutionary or taxonomic interest until the turn of the century when oat breeders, especially in Europe and Canada, began to take note of the frequent occurrence of plants with wild oat characteristics in fields of cultivated oats. Experimental work on them was then started in several countries. Fischer (50, 51) observed that "wild oats" found in fields of cultivated winter oats differed in panicle shape from true *A. fatua.* In this respect they were always like the cultivated variety in which they occurred. He found, as have many others since (*e.g.,* 11, 55, 87), that they do not have the characteristic "delay germination" of *A. fatua* which enables its seeds to lie dormant in the ground over winter or for longer periods. He considered these forms to be spontaneously occurring "reversions" or "throwbacks" towards the original wild type.

In Canada during the period 1909–1912, several workers (26, 31–34, 43, 64) showed that false wild oats, or fatuoids, occur in many varieties of oats and are like the variety in which they occur except for the one group of diagnostic characters; that they tend to germinate immediately after falling to the ground and hence to be destroyed by cultivation or winter-killing, with the consequence that they are not a serious weed-pest as are real wild oats with their capacity for remaining dormant; and, correlatively, that they do not appear to increase in frequency in oat fields observed over a period of several years. Newman (121) and G. H. Clark (The Editor of Criddle's 1912 bulletin) added to these conclusions the fact established by Nilsson-Ehle (124, 125) that the group of characters comprising the "fatuoid complex" is inherited as a unit. Further evidence for these conclusions has since been presented (12, 19, 22, 116, 122, 163) and is again given in the more detailed genetical studies to be reviewed in Section B of this article.

Nilsson-Ehle (124, 125) records that Hjalmar Nilsson, his predecessor at the Plant Breeding Institution, Svalof, Sweden, had noted towards the end of the last century the important fact, already referred to, that the "atavists" or "reversions" which he found in many varieties and lines of oats arose from the normal in two steps. The first generation plants differed from those of the parental strain only in having a stronger awn and a more markedly pubescent base on the primary grain of each spikelet. These plants segregated in the next generation to give forms like themselves, the parental cultivated form, and fully formed "atavists" with hairy disarticulation-surfaces and strong geniculate awns on every grain of the spikelets. These "atavists" were termed "fatuoids" by Nilsson-Ehle (130) who from extensive genetic experiments begun in 1900 concluded that they are "loss mutations", that cultivated oats have genes that mask or inhibit the effect of wild-type genes still carried in their genotype and that loss of these inhibitors permits the appearance of wild-type characteristics. Nilsson-Ehle's studies mark the beginning of the experimental work on fatuoids; they will be considered in Section B together with the work of his opponents, some of whom still uphold the theory that such fatuoids arise from natural crosses between *A. sativa* and *A. fatua.* In advance it may be said that much fruitless discussion would have been avoided if it had earlier been clearly realized that there are three classes of intermediate forms: *a*) those arising from natural crossing, *b*) the "heterozygous fatuoid" and other more or less similar forms now definitely known to be mutants, and *c*) the true-breeding "transition" forms of undetermined origin which provided the basis for the contention of systematists that *A. sativa* is derived by a progressive series of changes from *A. fatua.* These genetically diverse types sometimes cannot be distinguished without breeding tests, and some recorded tests made for this purpose have not been sufficiently comprehensive to give unequivocal results. Further confusion has arisen from the fact that while most workers, including the writer, use the term fatuoid as far as possible in the sense intended by Nilsson-Ehle who coined it, a few use it in its etymological sense to cover all fatua-like forms. The chief justification for the latter usage lies in the frequent indistinguishability of the fatua and fatuoid complex of grain characters when they are in the homozygous state. Nilsson-Ehle's usage involves consideration of other characters also. In practice it involves unanalyzed off-type plants being, at least provisionally, designated fatuoids if they appear to differ from the parental variety in only the one group or "complex" of characters, while those that differ in unassociated characteristics or otherwise give evidence of having arisen from crosses with *A. fatua* are termed fatua-like. The distinction, as will be shown, is difficult to maintain in some cases and may be impossible when both mutation and hybridization are involved, but without some distinction during analysis, only confusion results.

The speltoid complex of characters is distinguishable by experts from the analogous *T. spelta* complex, and less confusion has for this and other reasons arisen concerning wheat mutants.

Steriloids are mutant types that resemble the Mediterranean wild oat, *A. sterilis,* in their awn and glume complex of characters. They are found in varieties of both *A. sativa* and *A. byzantina.* Subfatuoid and semi-steriloid forms also occur in both of these cultivated "species". Their diagnostic characters are illustrated diagrammatically in Figure 1.

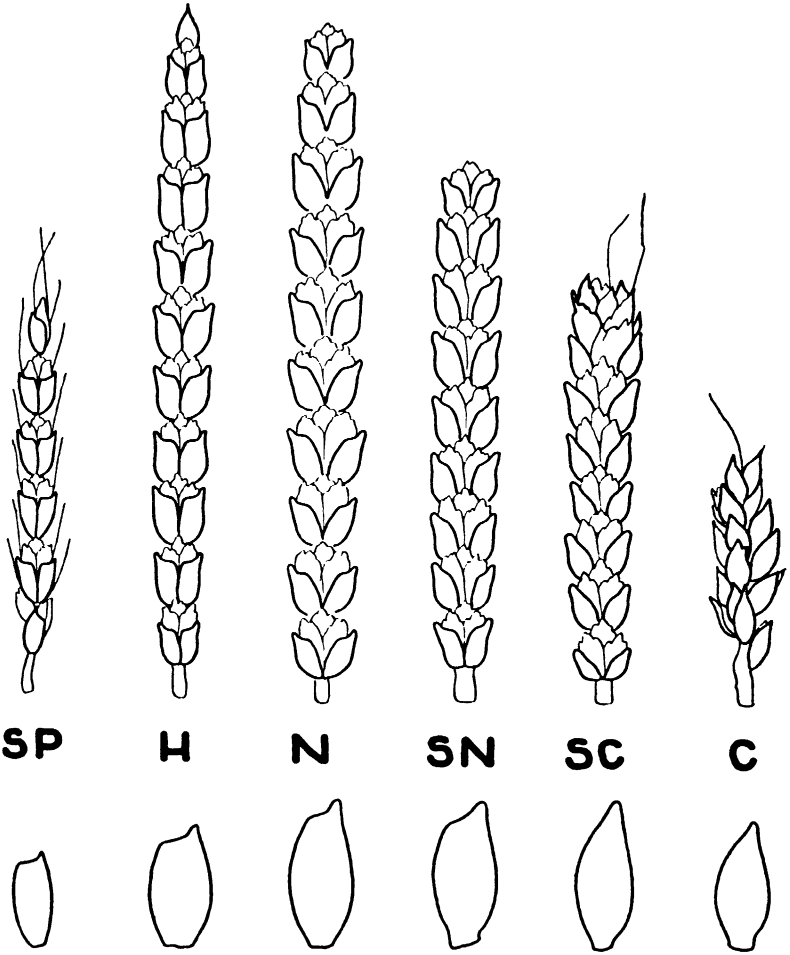

FIG. 2. Diagrammatic representation of the head shape, outer glume and awn characters differentiating speltoid (SP), heterozygous speltoid (H), normal (N), subnormal (SN), subcompactoid (SC), and compactoid (C) mutants of common wheat.

Wheats

Nilsson-Ehle (127) reported that in 1904 he had found in a winter variety of *T. vulgare* Vill.[2] some plants more or less resembling *T. spelta* L., which he designated "speltoids". Later they were found in many varieties and in the progeny of intervarietal and interspecific hybrids. Most were bearded, but a few beardless speltoids and a few "compact-headed" forms resembling *T. compactum* were also found. From the beginning it was evident that these forms were characteristically mutants, not natural hybrids or "transition forms". The extensive genetic studies since made of them will be reviewed in Section C. The diagnostic characters of the principal mutant types are illustrated diagrammatically in Figure 2.

Winge (210) discovered that various off-types of wheat are characterized by irregularities of chromosome number and behaviour. He related their occurrence to the polyploid nature of *T. vulgare* (see Section D). Shortly afterwards Goulden (57) and Huskins (65) found more or less similar irregularities in various fatuoid oats and attempted to correlate them with the origin and genetic segregation of these forms. In recent years most genetic studies of *Avena* and *Triticum* have attempted to relate breeding data to the polyploid chromosome constitution of these genera, and, despite obvious differences, many parallel phenomena have been discovered in them. Hence in Section D some of the recent genetic data on wheat and oats will be reviewed together along with cytogenetic studies of both. General reviews of the speltoid and fatuoid problems have previously been made (47, 76, 78, 119, 138, 168, 205).

B. GENETIC STUDIES OF FATUOID, STERILOID AND SUBFATUOID OATS

The origin of Fatuoids—the Mutation Theory

The experiments of Nilsson-Ehle (124, 125, 129) showed conclusively that the heterozygous fatuoids he discovered arose through mutation and that the fatuoids were segregates from them. They were clearly not derived from natural crosses between *A. sativa* and *A. fatua.*

Many of them arose in pure-line varieties of *A. sativa* in an area in which no *A. fatua* was known to occur. They resembled the variety in which they arose in all respects except the "fatuoid complex" of characters which in the "homozygous" form comprises a disarticulation callus or "sucker mouth" at the base of each grain of the spikelet, a tuft of hairs around this callus and on the rachilla, and a twisted, geniculate awn on each grain. This complex was found to behave as a unit in inheritance—crosses of true-breeding fatuoids with normal plants of the same variety gave an intermediate form which on selfing gave only normal, heterozygous fatuoid and fatuoid progeny, and these in ratios approximating 1:2:1.

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[2] *T. aestivum* L. under current International Rules of Botanical Nomenclature, but *T. vulgare* is the name almost universally used.

The mutation was sometimes found to occur repeatedly in the same pure-line of oats. When it occurred in the descendants of crosses between different cultivated varieties, Nilsson-Ehle found the usual segregation for many characteristics differentiating the original parents, but the fatuoid complex of characters behaved as a unit in inheritance. Occasionally plants were found with the heterozygous fatuoid base type but awns on two grains of some spikelets, instead of the characteristic one on the primary grains only. This seemed at first to be a break-up of the complex, but Gante (53), one of Nilsson-Ehle's students, found it to be a non-hereditary fluctuation. Nilsson-Ehle (130) noted, however, that Surface (169, 170) had found some indications of a break-up of the fatua complex in crosses of *A. sativa* by *A. fatua*. Furthermore, in speltoid wheats which appeared to be analogous off-types he had himself (127, 128) found mutations affecting only one part of the glume-awn complex. Nilsson-Ehle concluded, therefore, that though the fatuoid complex behaved as a unit-character in inheritance, fatuoids probably differ from their parent variety by a "complex" of several closely, or even absolutely, linked factors or genes. They and speltoids were therefore termed "complex mutations". In 1920 he had speculated that mutations affecting a whole complex of genes might be "deficiency mutations" involving loss of a segment of chromosome like those then recently discovered, and interpreted as such from genetic evidence, by Bridges (14) in *Drosophila.*

Many authors have since confirmed Nilsson-Ehle's observations and supported the mutation theory of the origin of fatuoids (*e.g.*, 4, 7, 8, 23, 24, 40, 54, 65–84, 88, 89, 109, 118, 133–137, 149, 150, 167, 168).

Fatuoids almost invariably appear first in the heterozygous ("het") form, but Marquand (116) reported one case of a "homozygous" fatuoid arising directly from the normal. Alabouvette and Friedberg (8) had four cases and Oescu (139) one. It is naturally very difficult to rule out entirely the possibility of error, such as accidental admixture of seed, but Alabouvette and Friedberg feel sure that at least one of their cases is beyond doubt. In some varieties of oats fatuoids arise with a particularly high frequency. Fulghum, a variety classed as *A. byzantina,* is one such, and Coffman and Taylor (24) state that since het. fatuoids arise in it with the frequency of one in every 125 plants, the fatuoid might be expected to arise directly from the normal once in $(125)^2 = 15{,}625$ individuals. This would, of course, be expected only if the mutation occurred equally often in male and female gametes (or somatically), and if both male and female mutant gametes function as readily as normal ones. As will be shown later, the latter condition, at least, rarely occurs. The true expectation under ordinary conditions is, therefore, considerably lower than one in 15,625. Derick and Love (42) X-rayed seeds of the Trelle Dwarf oat which is probably a chromosome mutant (Derick, unpublished). The appearance of the plants resulting from these seeds was not recorded in detail but 3% of their progeny were fatuoids.

Fatuoids have now been found in almost all varieties and "species" of hexaploid *Avena* excepting *A. fatua,* in which they would be phenotypically indistinguishable even if the fatuoid complex were not genetically identical with the fatua complex, as it appears to be —apart from associated modifiers. They occur frequently in intervarietal and "interspecific" crosses. They have not, however, to the author's knowledge yet been reported in a completely awnless variety of *A. sativa* long grown in Central U.S.S.R. and latterly at the University of Alberta and elsewhere (see 68). The vigor and fertility of fatuoids approaches that of the parental strain when the chromosome constitution approaches the normal—as it does in most fatuoids that are picked up in ordinary seed selection work. Fatuoids picked out in special searches are often chromosome-deficient types of low fertility and vigor.

The Natural Crossing versus Mutation Argument

Nilsson-Ehle had emphasized the absence of *A. fatua* in the area where he found his fatuoids, and similarly Newman (122) recorded that fatuoids occurred in Prince Edward Island where *A. fatua* was then unknown. Nilsson-Ehle also emphasized that fatuoids differ by several or many characteristics from *A. fatua:* Their lemmas are glabrous, not hairy, as in most forms of *A. fatua;* their panicle shape, grain shape and size and their germination are like those of the variety in which they arise; so, it may now be added, are other plant characters such as the ligules and the colour of the grains—with the partial exception of yellow (2, 89) which appears in some strains to be genetically linked to the fatuoid complex or its suppressor.

Nilsson-Ehle recognized, of course (125, 129), as did his supporters (*cf.*, *e.g.*, 163 and the discussion following Crepin's 1927 report), that among the segregates of natural crosses between *A. sativa* and *A. fatua* there will appear types that are like fatuoids with respect to the "fatuoid complex" of characters. But, whereas fatuoids differ from the variety in which they occur only by this complex, which segregates as a unitary difference, the fatua-like segregates of natural crosses differ in and segregate for many other characteristics also. Thus natural crossing of cultivated oats with *A. fatua*, which is not uncommon and may occur over considerable distances (25, 41), will give rise to "wild" forms that may in some cases be distinguishable from fatuoids only by very careful examination and possibly only by breeding tests. In most cases, experienced workers, by taking note of other characters of the grain or the plant, can distinguish between them at sight, and Åkerman and Bader (7) stress that the structure and inclination of the base of the grains is alone sufficient to differentiate heterozygous fatuoids from the F_1 plants of any of their own *A. fatua* × *A. sativa* crosses, as the normal sativa factor is less dominant over the fatua gene complex than over the mutant locus.

Nevertheless, the uncomprising view that all "transition" or "wild" forms found in fields of cultivated oats are the result of natural crossing with *A. fatua* is held by several authors. Possibly this attitude stems in some cases from the disinclination of the older systematists to accept the mutation concept in general. It may now be encouraged amongst taxonomists by the close relationship assumed for *A. fatua* and *A. sativa* in the modern classification of the Euavenae, since this permits a wide range of fertile forms being considered segregates of common ancestry. Thellung, the main author of the current polyphyletic system, supported Nilsson-Ehle in his earlier papers (175–177) but later reversed his views (178). Schultz (158), who is largely responsible for the polyphyletic system for wheat, believed Haussknecht's oat transition forms to be hybrids. Zade (211–213) collected especially vigorous intermediate forms with hairy glumes, which segregated cultivated, intermediate and wild types in a monofactorial, 1:2:1 ratio. The wild segregates had pubescent foliage-leaves and glumes. Zade considered them to be fatuoids, though they were clearly natural hybrids which did not fit Nilsson-Ehle's definition of fatuoids. Recently (see 7) Zade has become convinced that at least those fatuoids arising in established pure-lines of *A. sativa* cannot be due to natural crossing. Crépin (28–30) found natural hybrids between *A. sativa* and *A. fatua,* and from them, though they fulfilled the expectations set forth for such hybrids by Nilsson-Ehle, argued, with the support of Schribaux (157), against the origin of fatuoids by mutation. While Zade's and Crépin's observations were not on fatuoids and therefore do not give direct evidence on the origin of these forms, their studies and others (1) which will be detailed later, demonstrate clearly that fatua-like forms almost indistinguishable from mutant fatuoids may frequently be segregates from natural hybrids with *A. fatua.*

It is, however, difficult to appreciate von Tschermak's (185–191) prolonged attacks on the mutation theory of the origin of fatuoids. The weight of his position and authority has obviously influenced certain other workers, and there has been so much confusion of the issues involved that critical examination must here be given them. In so doing it will be necessary to emphasize and reiterate some of the elemental features of the problem. Tschermak reported that in the F_2 of *A. sativa* x *A. fatua,* there was an interacting two-factor segregation for the fatua-complex, this giving intermediates, sativa and fatua types in what he claimed as a 9:3:4 ratio (the actual numbers in the largest family were 444 intermediates, 132 sativa and 158 fatua). All other investigators of *A. sativa* x *A. fatua* crosses find a monofactorial, 1:2:1 ratio (1, 7, 8, 49, 54, 108, 110, 145, 169, 170, 214), though Florell (52) obtained a simple two-factor ratio of 12 byzantina: 3 sterilis: 1 fatua in the F_2 of *A. byzantina* var. Coastblack x *A. fatua.* A critical test of Tschermak's interpretation of his F_2 ratio is difficult because his F_3 material is too limited. Åkerman and Bader (7), however, grew a large F_3, and while on Tschermak's interpretation two thirds of the F_2 sativa types should have segregated in F_3, all those they tested bred true, as expected on the monofactorial scheme. The intermediates all gave sativa, intermediate and fatua types in a 1 : 2 : 1 ratio, whereas on Tschermak's two-factor scheme only two thirds of them should have given fatua segregates in F_3 and the remainder should have either bred true or segregated only sativa and intermediate types. There is thus no evidence from their extensive data favouring Tschermak's interpretation. His results seem to be due to his method of distinguishing between F_2 sativa types and intermediates on the basis of the dorsal pubescence of the glumes—a rather unsatisfactory characteristic (see 7, p. 15).

But Tschermak, extending his dubious interpretation of the *A. fatua* × *A. sativa* crosses to fatuoids (which he had not personally studied), argued further that they too are determined by the interaction of two independently inherited factors. If this were so, it would, of course, throw doubt on the mutation theory of their origin, since it would then be necessary to postulate that simultaneous mutation of two independent genes or gene complexes occurs not infrequently. Of his claim that the ratios obtained by various workers with het fatuoids were nearer to 9 : 3 : 4 than 1 : 2 : 1, it must be noted that he give no tests for significance, and that when Love (111) applied the χ^2 test to all the ratios cited by Tschermak it was clear that in only one case was his contention favoured. Actually there is nearly always a deficiency of fatuoid segregates and a corresponding excess of the sativa type (as was first shown long ago by Nilsson-Ehle), instead of the reverse expected on Tschermak's two-factor interpretation.

In its simplest form, as advocated by both Tschermak and Zade prior to 1918, the natural crossing theory of the origin of fatuoids ran into the insuperable difficulty, cited above, that whereas fatuoids are like the variety in which they arise except for the one complex of characters, the F_2 and later progeny of crosses between *A. sativa* and *A. fatua* segregate for or differ in many characteristics. To surmount this difficulty one would, as Nilsson-Ehle (130) remarked (translation), "be forced to the assumption, which cannot be called less than nonsensical, that white oat strains cross only with white *A. fatua,* yellow strains with yellow *A. fatua,* side-headed with side-headed and so on". In their later papers Zade (213) and Tschermak (190, 191) attempt to get around this difficulty by making the assumption that the natural crosses which they postulate as the source of Nilsson-Ehle's glabrous white fatuoids must have been with yellow or grey glabrous *A. fatua* (white is rare; the commonest are black and hairy) and must have occurred many generations previously. By shifting the origin back to earlier generations, however, they only open up a new problem: the old pure-lines of oats in which some of Nilsson-Ehle's fatuoids occurred must now be assumed to be concealed heterozygotes, apparently breeding true to type, but actually continuing to segregate. There is no evidence for their having such a heterozygous constitution in the usual sense of the term and Tschermak offered no details. Heribert-Nilsson (63), however, put forward an ingenious scheme to explain not only fatuoids but recessive mutations, in general, as arising through segregation in concealed heterozygotes. Though invalid as formulated (see 130) Heribert-Nilsson's idea remains an historically interesting speculation, since we know now that being amphidiploids or allopolyploids, both *A. sativa* and *A. fatua* are probably of ancient hybrid ancestry. In a special sense, quite distinct from that envisaged by Tschermak, chromosome aberrations occurring in polyploids may be regarded as a kind of internal segregation or recombination process. This must not, however, be allowed to obscure the real issue in the natural crossing versus mutation controversy over the origin of fatuoids or other mutants.

By 1922 fatuoids had been found in a very large number of cultivated varieties of *A. sativa,* and the regularity with which they resembled the diverse parental varieties in characteristics other than those of the fatuoid complex led Garber (54), who favoured the mutation theory, to emphasize that on the natural-crossing theory of origin, it would be necessary to postulate some mechanism for the elimination of all phenotypes other than those which are like the parental strain, apart from the single complex of fatuoid characters.

Aamodt, Johnson and Manson (1) considered this elimination possibility in detail. They had crossed black, pubescent *A. fatua* with a white, completely awnless, glabrous *A. sativa* (Zhegalov's Selection 76) which, as mentioned, has the distinction of never having produced fatuoids (see 68). From four F_1 plants they had grown an F_2 totalling 69 plants. These exhibited "practically all combinations between the parents in lemma-colour, awn development and pubescence" but only the three types of grain-base: fatua, intermediate and sativa. Fifty-four F_2 plants had black grains, eleven were grey and four were white. These white-grained plants comprised three with the intermediate base type and one with a fatua base. Their resemblance to heterozygous fatuoids and fatuoids, respectively, caused Aamodt *et al.* to call them "synthetic fatuoid types".

Coincidently these authors were studying "aberrant grain types found in threshed stocks and off-type plants selected in the field" from "elite" strains of Victory and Banner oats. Most or all of these appear to have been the products of natural crossing between *A. sativa* and *A. fatua*. Those that segregated produced populations "which corresponded more or less closely to certain types of segregation observed in the F_3 from the artificial cross".

Aamodt *et al.* were so impressed by this proof that many off-types which may commonly be classed as fatuoids are really the result of natural crosses, that they became "convinced that fatuoids arise through natural crossing between *A. fatua* and *A. sativa*", though they "admit the possibility of origin in other ways". From this standpoint they state that what "really needs to be explained is not the appearance of the fatuoid, but the more or less complete selective elimination of segregates other than fatuoids". "How is it", they ask, "that fatuoids are not found invariably in association with a host of black and grey off-types? The answer", they say, "is that fatuoids *have* been found with considerable regularity in association with these other aberrants. This is particularly true in fields grown from seed stocks which have not undergone selection for a considerable time". They recognized, however, that "in well-selected seed stocks, fatuoids are usually found unassociated with other off-types". To explain this they maintain that: "When selection is practiced in a field or plot, the black or grey segregates from a natural cross would be readily observed and removed, the homozygous fatuoid types would also be removed, but the heterozygous fatuoid type, similar in colour to the cultivated parent would, in many cases, escape notice and be propagated with the selected seed stocks".

Considering this suggestion, Åkerman and Bader (7) state (translation): "Naturally it is possible that in this way heterozygotes may arise which in habit, etc., are very similar to the normal type. And this is especially the case when the original heterozygotes are backcrossed to the normal type".

To the writer it seems that the suggestion of Aamodt *et al.* contains both an under- and an over-statement: the heterozygous fatuoid when in plots of awnless varieties is not very likely to be missed by competent selectors and, on the other hand, it would require exceeding skill to pick out in a field all gray-seeded forms, all with pubescent grains, or all liguled segregates in eligulate varieties, *etc., etc.* Aamodt and his collaborators themselves had great difficulty in scoring their plants for gray or white grain, even in the laboratory. They conclude an account of their difficulties with the remark that "it is impractical to distinguish between white and grey grain colour". One asks, is it not probable that the difference between field and plot stocks is that in the latter natural hybrids with *A. fatua* are most likely to be picked out and eliminated at the F_1 stage, whereas in fields they may be missed at their inception and so give F_2 and later generation progeny that in some cases resemble the parental cultivated variety in many or most respects, apart from the fatua complex? Confusion of fatuoid-like hybrid segregates with true mutant fatuoids would therefore be much more likely to occur in field than in plot selection.

In any case it is unfortunate that Aamodt *et al.* did not emphasize that their "synthetic fatuoids" were classified as such only on three characters of the their grains—the fatuoid or het fatuoid base with white and glabrous lemmas. They were recombinants that on the basis of grain characters alone might well have been classified as fatuoids by any seed inspector of threshed grain who naturally would have no other basis of classification. Hence, the observations have great practical significance for seed growers and inspectors, especially in western Canada where *A. fatua* is a common weed and natural crossing with it takes place much more readily than in many other regions. The "synthetic fatuoids" were, however, definitely not fatuoids according to the generally accepted definition of the term. Seeds which Professor Aamodt and Dr. Johnson kindly supplied to the writer produced plants that differed from the parental variety of *A. sativa* in growth habit, pubescence of the foliage leaves, date of maturity and many other less definite characteristics. This work is, then, very significant for the practical agricultural problem of the origin of off-types in cultivated oats, though not directly for the problem of the origin of true fatuoids. One may note, in stating this conclusion, that Åkerman and Bader (7), who examined several thousand F_2 and F_3 segregates of *A. sativa* × *A. fatua,* were unable to find any two identical with each other or with the parent variety when all plant characteristics were considered—this despite the fact that their crosses were with a glabrous *A. fatua.* Further, as Nilsson-Ehle originally pointed out and Åkerman and Bader have re-emphasized, the base of het fatuoid primary grains is characteristically very like that of the parental *A. sativa* strain, whereas heterozygotes of *A. sativa* × *A. fatua* have a more intermediate base-type. It seems, then, that fatuoids can often be distinguished from segregates of *A. sativa* × *A. fatua* by grain characters alone and can invariably be distinguished if all plant characters are considered. It should of course be borne in mind that the fatuoid mutation could occur in descendants of *A. sativa* × *A. fatua.* In that case the resultant true or mutant fatuoids, on account of their having some *A. fatua* plant characteristics, would probably be classed as natural hybrids. Error can invade from either side! In areas where natural crossing of cultivated oats with *A. fatua* is not infrequent, the combination of hybridization with mutation may produce complicated situations almost incapable of resolution. The complexity of the polyploid genetic and chromosomal constitution of *A. sativa* is, of course, directly related to its capacity to produce fatuoids by "loss mutation".

Recently, Taborda de Morais (171–174) has criticized the supporters of the mutation theory, particularly Alabouvette and Friedberg (8), and accused them of neglecting the possibility of natural crossing of *A. sativa* with glabrous wild oats such as *A. fatua* var. *glabrata* Peterson or var. *vilis* (Wallr.) Haussknecht. He claims that such crosses would result in F_1 plants phenotypically identical with *A. sativa* but segregating the wild parental type in F_2; these latter would then erroneously be called fatuoid mutations. Actually, Zhegalov (214), who has made these crosses, was well able to distinguish the F_1 of *A. sativa* × glabrous *A. fatua* from the *A. sativa* parent and also from het fatuoids by grain characters alone (see Figs. 54 and 55 in 115). The strength of the F_1 awn depends, like that of het fatuoids (125), on the *A. sativa* parent, but even in crosses of the completely awnless variety Zhegalov's Selection 76 with *A. fatua,* awns distinguish the hybrid (1). Badly strained already, Taborda de Morais' hypothesis runs into seemingly insuperable difficulties when plant characters other than those usually considered important by taxonomists are taken into account, for example, only eligulate fatuoids arising in eligulate pure-lines of *A. sativa* (54, 89, 163). The decisive comment (130) quoted above, on the not less than non-sensical assumptions that have to be made to fit the simple formulation of the natural crossing theory to the origin of fatuoids, seems as appropriate here as to the earlier variants of the argument.

Chimeras or Mosaics

The most striking evidence against the natural crossing hypothesis for the origin of fatuoids is found in the occurrence of "chimeras" or "mosaics", *i.e.,* plants bearing both normal and fatuoid grains. The diagnostic features of fatuoids are glume characters composed, of course, of maternal tissue; the occurrence of two or more distinct types of grain on a single plant can not, therefore, be due to natural crossing. Chimeras must be due to mutation, using this term in its broader sense. The possibilities to be considered within the scope of this term are: (*a*) gene mutation, (*b*) chromosome aberration, (*c*) somatic segregation, and (*d*) various aspects, combinations or complications of these. Chromósome aberration may be considered a type of somatic segregation; chimeras may result from somatic crossing over in hybrids. But these, again, are distinct issues from those raised by the natural crossing hypothesis for the origin of fatuoids. Care must also be taken to see that supposed chimeras differing in entire culms are not really twin seedlings.

Tschermak (186–190) found one mosaic spikelet in the F_2 of an *A. sativa* × *A. fatua* cross. It had a brown, hairy, strongly awned fatua-type primary grain and a white, glabrous, weakly awned, sativa-type secondary grain. The fatua grain gave a fatua plant, and grains from neighboring sativa spikelets gave plants which (translation) "were all sativa forms and varied only in awning and hairiness of the base of the glumes". Similar mosaic spikelets were described by Tschermak in crosses involving different strains of *A. sativa.* It seems most likely that all his chimeras originated on plants heterozygous for the fatua complex—a possibility which he admits but discounts. Chimeras in oat species hybrids have been found by others (*e.g.*, 15, 86, 148). Dr. H. Hunter (unpublished) analyzed a fatuoid-normal chimera found at Cambridge, and Huskins (70) described a complex one bearing normal, steriloid ("semi-fatuoid") and fatuoid grains. Another (80) bore fatuoid, subfatuoid and steriloid grains.

Diverse Ratio-types

The fatuoids discussed above are all of the type that gives a 1 : 2 : 1 ratio in F_2 when crossed with the normal type, and the het fatuoids from which they have arisen do the same when self-fertilized. For many years this "Series A" was the only ratio-type known, though three distinct ratio-types (roughly 1 : 2 : 1, 1 : 5 : few and 1 : 1 : few) designated "Series A, B and C" (128) had long been known in speltoid wheat. (These Series are here designated α, β, γ to avoid confusion with chromosome symbols—see Sections C and D). Amongst the het fatuoids from grains selected out of threshed samples of Banner oats by Goulden (57), however, was one that gave 20 normals, 15 het fatuoids and 5 fatuoids, of which two were dwarf. This is Series γ. Another het fatuoid gave 0 normals, 12 het fatuoids and 18 dwarf sterile fatuoids. This has now been designated "modified β Series" for reasons that will be given later. Nishiyama (133) obtained a strain of this type from a Series α strain supplied to him by the writer who has repeatedly found the same changed type in this strain. A het fatuoid plant found in a head-selection plot of Victory oats (69) gave seven normal, six het fatuoids and one sterile, dwarf fatuoid. In later generations involving many thousands of plants, het fatuoids of this strain have given these three types of progeny in ratios of about 1 : 30 : 1. They are classed as Series β, though the proportion of heterozygous progeny is much larger than in Nilsson-Ehle's Series β speltoids. A het fatuoid of the variety Kanota that gave nearly a 1 : 2 : 1 ratio, but with the fatuoid progeny dwarf and almost sterile (69), has since predominantly given normal and het fatuoids in more or less equal numbers and only a small proportion of dwarf fatuoids. It is Series γ, but sub-strains which must be classed as Series β continuously arise from it, as they do from Series γ het speltoid strains. Other variations of the basic α, β, γ Series ratio-types have also been found.

The ratio-types of fatuoids are at present more diversified than those of speltoids. But Series α remains by far the commonest type of fatuoid, whereas the three Series of speltoids apparently occur about equally often. It appears evident, however, that this is due more to the way the mutant forms are collected than to any difference in their frequency of occurrence. To get any idea of the frequency of origin the mutants must be discovered in the first generation, which is almost invariably the heterozygous form.

Het speltoids, being taller than their normal wheat sibs as well as distinctive in head-type, are easily noticed, while het fatuoids are not taller than normal oats and are not very easily picked out in the field. Fatuoids are much more easily noticed than het fatuoids, but speltoids not much more easily than het speltoids. When fatuoids or speltoids are picked out in a field they are usually of Series α because in Series β or γ the "homozygous" segregates are almost always dwarf and more or less sterile, and they usually fail to reach maturity when crowded.

There is another major difference affecting the frequency of the different Series in oats and wheat: fatuoids are very often picked out of threshed grain. Their progeny are then usually of Series α, since fatuoid seeds of Series β and γ are mostly sterile. Het fatuoids are rarely picked out of threshed samples of oats, for, while the primary grains of het fatuoid spikelets can usually be distinguished from normal grains, the secondary and tertiary grains can not. Speltoids and het speltoids alike are always picked up as plants or heads, since, being devoid of glumes when threshed, their grains can not be distinguished from normal ones. Further, speltoid or fatuoid strains originating in mosaic heads *i.e.*, by somatic mutation, are usually of Series β or γ, and mosaic heads of wheat are much more easily noticed than mosaic oat panicles.

It follows from the above that the fatuoid strains collected in the ordinary course of seed selection or improvement work are most likely to be of Series α, regardless of the frequency with which the different types originate, while for wheats the frequency of discovery, especially in well-rogued stocks, may be in some appreciable measure an indication of the relative frequency of occurrence of the mutations producing the different Series. On this latter point exceedingly little information is available for oats. Neglect of such considerations has led to several unfortunate controversies and misunderstandings.

Steriloids, Subfatuoids and Allied Off-types

Besides fatuoids there are off-types which in varying degrees resemble *A. fatua* or *A. sterilis* in basal articulation and awning. In *A. sativa* var. Sixty Day one off-type was found that "differed markedly from the normal fatuoid, in that its seeds were not dropped immediately on ripening and a decided tendency existed in many spikelets for the florets to remain together in threshing as is true with derivatives of *Avena sterilis*" (168). Huskins (70) obtained a strain of "semi-fatuoids", later called "steriloids", from one of a number of fatuoid-type grains found in a threshed sample of *A. sativa* var. Abundance. This steriloid type has "twisted and geniculate awns on both the first and second grains of each spikelet", but not on tertiary grains. "The primary grain has a medium-sized 'sucker-mouth', but the second has a solid base—like normal *A. sativa*". This off-type is, therefore, superficially like *A. sterilis* in awning and spikelet articulation, but the floret articulation is more like that of *A. sativa*, and in general plant habit and appearance it is typical *A. sativa* var. Abundance. Other more or less similar steriloids have been found in various varieties (19, 28, 29, 141, 145, 163). One in Kanota oats has been analyzed cytogenetically (80).

Jones (89) found a sub-fatuoid in an F_3 of *A. byzantina* x *A. sativa*. It has a fatuoid-like sucker-mouth on the primary grains, but the secondary and tertiary grains have only a partially developed disarticulation-surface and hence tend to remain attached. In some panicles the cleavage plane of the secondary grains was more rudimentary than in others, and there was "fracture of the rachilla at its base, as in *A. sterilis culta*" (*A. byzantina*). Pubescence of the sucker mouth and rachilla was like that of typical fatuoids. There were awns on all grains of the spikelets, but those of the fourth grain were very weak (personal communication from Dr. E. T. Jones—the fourth awn does not show in his published illustration). A "sub-fatuoid" found by the writer in the variety Kanota is very similar except that its fourth grains are awnless. (80). It may be significant that the variety Kanota has probably mixed *A. sativa* and *A. byzantina* ancestry.

Other off-types, too, have strongly developed awns associated with basal articulation nearer that of normal *A. sativa*. Many were found in *A. byzantina* var. Burt (21). Jones (89) described four distinct types from varieties of *A. sativa* or from hybrids of *A.*

sativa x *A. byzantina*. His and other analyses show that there is consistently a genetic association between base type, pubescence and awns, and it is indicated that many diverse forms behave as if they were members of a single allelomorphic series. It should perhaps be emphasized that no one has yet reported a hexaploid oat with fatua-type base but lacking awns. On the other hand, Jones (91) has shown that base and awn characters are independently inherited in diploid and tetraploid oats.

Amplifications of the Mutation Theory

In 1911 Nilsson-Ehle concluded that fatuoids arise through the loss of inhibiting factors which in normal *A. sativa* suppress the fatuoid factors that are always present in its genetic make-up. Jones (89) extended this interpretation to all the various off-types which, he showed, behave as if due to allelomorphs or a series of mutations on one chromosome. He considers the *fatua* or fatuoid to be the basic type and the cultivated form to be due to epistatic factors, on the "C" chromosome, which restrict the action of the primary awn and articulation genes. The mutations are changes of varying complexity which affect the epistatic factors, not the primary, wild-type genes. This concept is in general conformity with that adopted (69, 210) from cytogenetic evidence. It will be further developed in Section D.

Nilsson-Ehle had shown further that the degree to which fatuoid factors find expression in the heterozygote depends upon other independent factors (specific modifiers) present in the different cultivated progenitors. Many others (*e.g.*, 89, 145) have found modifying factors to be of very frequent occurrence and great significance in the expression of awn, pubescence and base type.

Some (7, 126, 149) have shown that the expression of the epistatic factors for the cultivated grain type is greatly affected by moisture and other environmental conditions, but that in the absence of these factors the hypostatic ("primary") fatua or fatuoid gene complexes are very stable in their expression.

Since Nilsson-Ehle's original mutation hypothesis for the origin of fatuoids is upheld by the evidence examined above, we may well conclude this section with a translation of the concluding paragraph of his 1921 (130) paper. This not only clarifies, and in the writer's opinion settles, all the older aspects of the problem, but embraces also the question still at issue—the nature of the mutation which produces fatuoids:

"The assumption must therefore be maintained that my fatuoids arise through mutation occurring in lines [of oats] which are homozygous in so far as the characters in question are concerned. As I stated in 1911, it is naturally not denied that fatuoid-like types can also arise through natural crossing with *A. fatua.* Indeed, cases are known where similar types can arise through either crossing or mutation. The fatuoids, segregating out of heterozygous fatuoids, that I have found in Sweden, are without exception mutations.

"Of course in saying this nothing is said about the cause or the nature and origin of the mutation. I, at least, do not imply in the concept of mutation anything but that through it a spontaneous hereditary alteration is produced which according to all the facts known to date has nothing to do with hybridization or segregation from hybrids. In what way, that is, by what change of the hereditary substance, the mutation arises is still an open question. If one speaks of the dropping out or loss of an hereditary factor every geneticist will understand that this is a purely formal expression which stresses the difference between the two members of a pair of allelomorphs and designates the alteration of A to a, but naturally does not say anything about the real internal process of change.

"But if anyone wishes in a wholly general way, and without considering the factual material against it, to ascribe mutations such as the ones described above to the consequence of hybridization, that is an inadmissable simplification of the situation which only obscures the problem and takes us further from a better understanding of it".

C. GENETIC STUDIES ON SPELTOID AND COMPACTOID WHEATS

Beginning in 1904, Nilsson-Ehle (127–129) studied speltoids from many varieties of *T. vulgare.* They arose as heterozygous forms and gave various segregation ratios. The first were found occurring in small numbers in a "square-head", dense-eared, variety and were distinguishable from it in the field by their longer, looser ears, longer stems or culms and later ripening. On mature heads the outer or empty glumes were shorter, almost square across the top, had stronger keels and bore green stripes running far down towards the base. Following self-fertilization the progeny of het speltoids comprised about equal numbers of het speltoids and normal plants typical of the original variety. Crosses of het speltoids as female with the normal as the male parent gave a similar 1 : 1 ratio, while the reciprocal cross produced only normal progeny. Evidently, ovules bearing either normal or speltoid factors were produced and functioned equally often, but pollen bearing the speltoid complex was either not formed or did not function in this strain.

Het speltoids arising in other varieties differed less from their normal parental type. Some of them gave speltoid ("Sp") as well as normal ("N") and het speltoid ("H") progeny in ratios of about 3N : 5H : 1.5Sp. In these, speltoid-bearing pollen evidently functioned, though less frequently than normal pollen. In some strains the speltoid segregates were bearded and in others beardless. Other het speltoids were found later that gave only normal and het speltoid progeny in a ratio of about 1N : 5H.

The characteristics distinguishing het speltoids from normal *T. vulgare* are further accentuated in speltoids; their outer glumes are short, square across the top, very strongly keeled and highly indurated. When ripe they can not be bent away from the spike without breaking. The spikes are long and slender, *i.e.,* the spikelets are far apart.

The height and vigor of speltoid plants varies with the Series (see below) to which they belong. Vigorous speltoids are often confused with *T. spelta* and *vice versa,* but, as Nilsson-Ehle pointed out and Watkins (204) has since emphasized in detail, there are, usually at least, significant minor differences. The rachis of *T. spelta* is brittle and that of speltoids characteristically not. The keel of *T. spelta* glumes is gently curved, while that of speltoids is straighter and has a sharp "collar" at the junction with the rachis.

Classification of the Ratio-types

In 1920 the speltoids found by Nilsson-Ehle (128, 129) were classified into three series according to the type of segregation of their self-fertilized heterozygous form:

"Series A" het speltoids were those giving normals, het speltoids and speltoids in ratios ranging from almost 1 : 2 : 1 to nearly 1 : 1 : 0. The deviations from 1 : 2 : 1 were explained, after reciprocal crossing tests, as due to varying degrees of failure of speltoid-bearing pollen to function in fertilization. This was confirmed by Åkerman (5) who found that in Series A the cross speltoid × het speltoid gave mainly het speltoid progeny (28H : 8Sp) and normal × het speltoid gave mainly normals (126N : 22H), while het speltoid × normal gave equal numbers (25 : 25) of het speltoid and normal progeny.

"Series B" het speltoids were defined as those giving a great excess of het speltoid yet few or no speltoid progeny (1N : 4 or 5H). Reciprocal crosses showed that their speltoid-bearing pollen almost never functions. They have also, Nilsson-Ehle shows, the additional complication that speltoid-bearing female gametes must be produced in great excess.

"Series C" het speltoids, on Nilsson-Ehle's original classification, produce slightly more normal than het speltoid progeny and very few or no speltoids. Non-functioning of speltoid-bearing pollen and the production of slightly more normal than speltoid-bearing ovules were held to account for this progeny ratio. Nilsson-Ehle had difficulty in deciding whether to assign some strains to Series A or to C, owing to the deviation of individual progenies. It is evident that zygotic elimination of het speltoids could play some part in determining the fluctuations and allow an excess of normals when plant mortality is high. In the studies of the writer and his associates all speltoids that give ratios close to 1N : 1H, with very few or no speltoids, are classed as Series γ, (α, β and γ being used in place of A, B and C, as with oats, to avoid confusion with chromosome symbols) and only those that produce an appreciable number of speltoid progeny of nearly or quite normal vigor are classed as Series α. This fits better the combined genetic and cytological data. It should, however, be stressed that there are intergrading types and that it now appears probable that the distinction between Series α and γ speltoids is one of degree only.

Nilsson-Ehle found that the degree to which the speltoid segregates differed in vigor from the normal was correlated with the degree of elimination of speltoid-bearing male gametes. Thus the relatively numerous speltoid segregates from Series α het speltoids are vigorous plants, the relatively rare ones from Series γ are weaker and the still rarer speltoids of Series β are weakest of all. Natural crossing, which is usually by normal pollen, occurs frequently in het speltoids which have weak speltoid progeny because they are genetically unbalanced types and have much sterile pollen. It occurs rarely in those which have strong speltoid offspring because they produce abundant good pollen.

Series α, β and γ het speltoids can all originate directly from the normal, and by 1921 each had been found five times by Nilsson-Ehle. Series α usually remains constant in ratio-type, but as many as 12% of the het speltoid progeny of Series γ het speltoids have changed to Series β. Nilsson-Ehle (129) and Lindhard (105–107) obtained Series γ from Series β in a few cases, but the possibility of this being due to natural crossing by pollen of γ strains was not ruled out. Subcompactoids occur as rare but fairly regular segregates from Series β het speltoids.

In Nilsson-Ehle's het speltoids which arose in pure-line varieties the segregation was confined to one complex of characters; they could not therefore be the result of natural crosses with other varieties or species. They occurred repeatedly, though infrequently. In most respects speltoid mutants parallel closely the fatuoid mutants of oats. Just as the latter are similar to but not identical with *A. fatua,* so also speltoids do not conform completely to the type of *T. spelta.* Nilsson-Ehle classed speltoids, like fatuoids, as "complex mutations", *i.e.,* as mutations affecting an hereditary complex consisting of several genes, and here also he considered the possibility that they might be due to deficiency of a chromosome segment.

Vestergaard (201) found a Series γ het speltoid and noted that the flowers of plants which are sterile from either genetic or environmental causes may remain open for more than a week and that if they become fertilized it is usually by pollen from nearby normal plants. Lathouwers (99–102) analyzed the complicated segregation of several "speltoid-like" plants found in pure lines of *T. vulgare* and showed that they had arisen through natural crossing with *T. spelta.* Later (103) he found what he believed to be a true speltoid within a partly sterile progeny. It gave indications of having arisen through chromosome aberration. The "speltoids" found by Ducellier (44) were probably natural hybrids of *T. vulgare* and *T. spelta.*

Lindhard (105–107) studied in Denmark more than 100,000 descendants of a single het speltoid that appeared in 1914 in a squarehead variety of *T. vulgare.* The original het speltoid gave 18 normal ("squarehead"), 54 beardless het speltoid and two bearded het speltoid progeny. Lindhard suggests that the bearded het speltoids may possibly have resulted from natural crossing; throughout his experiments bearded plants appeared sporadically. If the original plant was a Series β het speltoid it gave an unusually high proportion of normal progeny. In the light of later cases, it is here suggested that it was possibly a periclinal chimera, *i.e.*, one in which the outer cell layers were entirely het speltoid but the internal tissue, including some parts of the germinal layer, was in part composed of normal cells. With few exceptions the lines comprising the later generations of this family were Series β with the ratio of normal and het speltoid progeny ranging from 1:5 to 1:8. The normal plants were shown usually to be more viable than the het speltoids, and winter-killing, which was often very severe, apparently affected the ratios. Lindhard considered that a true ratio of 1:8 could, by zygotic elimination of het speltoids, be distorted to 1:5. In a few exceptional lines the normals were weaker than the het speltoids. One het "speltoid" that was obviously a natural hybrid gave a ratio of 1: 12.

In the first four self-fertilized generations from the original het speltoid plant no speltoid progeny were obtained. In Series β the speltoid segregates are ordinarily very weak plants, and as mortality was very high in Lindhard's cultures they were probably eliminated either during the winter or at any rate before heading. In the fifth, sixth, seventh and eighth generations speltoid segregates were obtained in a few lines. Some of these were the dwarf, more or less sterile plants characteristic of Series β. Others were vigorous and fertile and were always associated with a marked change in the segregation ratio of their parent het speltoid. It now appears probable that natural crossing was concerned in their origin. They will later have to be considered further, as they were apparently the progenitors of the plants in which Winge (210) found most of the multivalent chromosome configurations on which he based his theory of the origin of speltoids. Throughout Lindhard's studies bearded het speltoids and beardless speltoids occurred sporadically. Some may have been new mutations, but others were probably due to natural crossing, which was apparently a serious complicating factor. Lindhard (105) recorded a "primary" speltoid mutation rate of 0.2%. In Japan Ichikawa (85) found 0.08% to 0.3% mutation from the normal type to speltoid.

Speltoids Arising from Hybrids

Kajanus (92–94) found that speltoids comprised 0.3% to 1.9% of the F_2 progeny of various intervarietal and interspecific hybrids. He concluded that they arise through some form of internal segregation. Leighty (104) found 1% to 3% in some intervarietal hybrids but none in others. Watkins (202–207) has analyzed in great detail the pentaploid hybrid *T. turgidum* x *T. vulgare* from which speltoid-like types are regularly segregated. His conclusion of present interest is that speltoids arise from pure *T. vulgare* by a process of internal segregation made possible by polyploidy. This was first suggested from cytological evidence by Winge (see Section D).

Chimeras or Mosaics

Åkerman (3, 6), Kajanus (93, 94) and others have analyzed vulgare-speltoid chimeras. Some of these are plants with distinct normal and het speltoid culms—the roots have to be examined very carefully to make sure that they are not really twin seedlings; even then the possibility presumably remains that fusion of tissues might have occurred. In others a single head bears both normal and het speltoid spikelets, the line of differentiation usually being lateral. The diagnostic characters, being in maternal tissue, give no indication of the genotype of the grains borne on these heads. Sometimes grains from both parts of a chimera produce only normal offspring. Evidently in these cases the epidermal tissue but not the germinal layer has been affected by somatic mutation. In other cases the grains from normal sectors produce only normal plants and those from het speltoid sectors give both normals and het speltoids, showing them to be germinally het speltoids also. A speltoid developed from a seed produced in a het speltoid sector on one of Åkerman's chimeras. Het speltoid plants developing from the grains of a chimera often belong to Series β, but the immediate progeny-ratio of a chimera is naturally not significant for determining the Series, as it depends entirely upon the extent to which the mutation affects the different tissues of the parent plant. Lindhard (105) and Ishikawa (85) found "het speltoid-subcompactoid" chimeras in *T. vulgare,* and Nilsson-Leissner (132) found "spelta-vulgare" chimeras in the progenies of crosses between *T. vulgare* and *T. spelta.* Nilsson-Leissner's chimeras were usually heterozygous spelta with a vulgare epidermal sector. We may smile at 17th and 18th Century reports of mosaic wheat heads bearing oat spikelets (see 215) but one can not help wondering how men like Gerard, Cooke and their contemporaries came to give such particular accounts of them. Could the "oat" spikelets have been somatic mutations to a *T. polonicum* type of glume, perhaps from *T. turgidum* which, like *T. polonicum,* is a tetraploid species? *T. turgidum* has long been widely grown in England, and *T. polonicum* type glumes occurring sporadically on heads of it could have been mistaken for oat glumes if this latter species was then unknown there.

Numerous speltoids, many of which are chimeras, have been obtained after irradiation, aging, centrifuging and other treatments of *T. vulgare* seeds (17, 18, 37, 38, 39, 153, 159), and others. These mutants were for the most part chromosomal aberrants. Nilsson (123) obtained some indication that the physiological upset caused by bunt (*Tilletia*) may induce speltoid mutations, and Mr. E. S. McFadden, U.S.D.A., has evidence that frost during the flowering period may induce them (personal communication).

Part Mutations

The complete speltoid mutation involves the appearance of awns (which in wheat are a prolongation of the tip of the flowering glume or lemma) as well as modifications of the empty or outer glumes and of the rachis, which are described above. But beardless speltoids and bearded and half-bearded normals also arise from ordinary so-called "beardless" wheats. "Beardless" is here used in its popular sense to describe the common type of wheat which should strictly be classed as "tip-awned" (see 208). Truly beardless varieties of wheat are rare. Nilsson-Ehle called the bearded speltoids "total-mutations" and the others "part-mutations". At first he thought that the part-mutations were relatively infrequent and that their appearance was secondarily related to the total speltoid mutation. In a later study (131), however, he reported that the part-mutations are not at all infrequent and that in one strain they appear unaccompanied by bearded speltoids.

In bearded speltoids that arise directly and at one step by mutation there is complete linkage between awns and speltoid glumes: In crosses with beardless normals only beardless normal, beardless het speltoid and bearded speltoid progeny appear in F_2; the occasional appearance of bearded het speltoids is due either to natural crossing or to a new mutation or aberration (see later), not to ordinary crossing-over. In back-crossed interspecific hybrids (*T. turgidum* x *T. vulgare*) x *T. turgidum,* Watkins (204) found about 39% crossing-over between awns and glume characters. In crosses between a beardless speltoid found in Panzerweizen and the parental stock, which is "bearded normal", Nilsson-Ehle's data (131) show about 23% crossing over (his analysis is in terms of gametic ratios). The bearded speltoids that arose through crossing over are vigorous plants. In the F_2 of a "half-bearded" normal x beardless speltoid, Nilsson-Ehle notes that the characters bearded, half-bearded and beardless behave as if they were allelomorphs in that whichever pair are crossed together only two types of progeny occur in F_2 and these in a 3: 1 ratio. He points out, however, that the difference in linkage values, which appears to be significant, indicates that they cannot be due to simple multiple allelomorphic genes. He concludes that the bearded character in part-mutants is itself due to a complex of genes which are completely linked, as are the beard and glume genes (or gene complexes) of the total speltoid mutation; bearded, half-bearded and beardless simulate a multiple allelomorphic series of single genes because the mutation by affecting an interstitial segment prevents crossing over between the component factors of the bearding complex. It will be seen later how strikingly these concepts from purely genetic data can be fitted into the cytogenetic interpretation derived from later studies —hence this extensive consideration of Nilsson-Ehle's data and conclusions.

Compactoids, Subnormals and Associated Types

There remain for consideration here those mutants of *T. vulgare* which resemble *T. compactum.* These arise in the heterozygous form variously termed "compactum heterozygote", "subcompactum" or "subcompactoid". For the most part they occur as rare but regularly appearing offspring of Series β het speltoids. Lindhard (105, 106) found 184 in a total of 39,146 progeny from such het speltoids, and Nilsson-Ehle obtained 24 in a total progeny of 2,830. They also occurred twice in the progeny of Series γ het speltoids that had just changed over from Series β. They occur also, though very rarely, as mutants directly from *T. vulgare* and as sporadic segregates from intervarietal or interspecific crosses involving *T. vulgare* (see 13, 45). They have never arisen from speltoids.

Subcompactoids regularly segregate still denser-headed types termed "compactoids". Distinction between subcompactoids and compactoids can at best be made only within individual strains, as the former may be denser in one strain than the latter in another. In general, the term "subcompactoid" is used for the regularly segregating types, and "compactoid" for their denser-headed segregates which can breed true, though owing to chromosome anomalies and natural crossing they often do not.

Subcompactoids may be grouped into three main segregation types. One, the simplest, gives only normal, subcompactoid and compactoid progeny. Another, the rarest type, gives in addition a small and irregular proportion of het speltoid offspring. A third, the commonest but most complex type, gives many het speltoids and also "square-head heterozygotes" or "sub normals" in addition to normals, subcompactoids and compactoids. The ratios are irregular and could not be understood until the chromosome constitution was determined (see Section D). Nilsson-Ehle (128, 129) devised various schemes involving partial heterogamy to explain the peculiar ratios, but later (131) stated that they had all been proved invalid. Lindhard (107) attempted an explanation on the basis of a pair of factors, L and l, which affect both the ear density and the progeny ratio. Since grossly abnormal chromosome behavior is now known to be involved, it is not surprising that these efforts, lacking cytological data, met with little success.

Lindhard's Series β het speltoids produced a number of other aberrant forms besides subcompactoids. These included: (*a*) a "perennis" type that produced a dense rosette of leaves, only rarely formed heads, and these very late and almost sterile; (*b*) a dwarf normal type; (*c*) a dwarf het speltoid; and (*d*) a very late-maturing type. The latter, "Spät Kolben", segregates its kind, and the perennis type in about equal numbers. The dwarf normal, "Zwerg Kolben", segregates its type and square-headed normals in either 1:1 or 1:4 ratios.

D. CYTOGENETIC STUDIES OF MUTANT WHEAT AND OATS

The genetic complexities outlined in the previous sections have largely been clarified by various cytogenetic studies dating from

1924 to the present. Cytogenetic analyses of *Triticum* and *Avena* which bear on the general problem may here be considered together, as they are in many respects parallel and in others complementary. Each genus has, of course, special cytogenetic features, and these are taken up in detail in the various papers cited and those of the writer and his associates now in press.

After many erroneous chromosome counts by earlier workers (nine had reported $2n = 16$ in *T. vulgare,* and as high as $2n = 48$ had been recorded in *Avena*), the correct numbers for *Triticum* were determined in 1918 (151, 154, 155) and for *Avena* in 1919 (96). Each genus contains three groups of species which are numerically diploids, tetraploids and hexaploids having $2n = 14$, 28 and 42 chromosomes.

It should be stressed that though *T. vulgare* and *A. sativa* are numerically hexaploid, their 42 chromosomes almost invariably form 21 bivalents and their genetic behaviour is correlatively like that of a true diploid in most respects. They are allopolyploids or amphidiploids which have doubtless arisen through chromosome doubling following interspecific hybridization (180). The ways in which such amphidiploids may differ from true diploids have been discussed by many authors including Stadler (166) and the writer (72) on cereals.

Winge (209) was one of the first to recognize the importance in plant evolution of chromosome doubling following hybridization. He was, in consequence, in a position to appreciate fully the cytogenetic possibilities inherent in *T. vulgare* when its polyploid nature was revealed and to speculate profitably, even though, in the light of subsequent discoveries, not always very accurately, on the significance of the aberrant chromosome numbers or behaviour that he found in various wheat mutants. Winge (210) reported that in speltoids obtained from Lindhard the normal chromosome number was present but that 19 bivalents and one quadrivalent ($19_{II} + 1_{IV}$), instead of 21_{II}, were occasionally formed at meiosis in pollen mother-cells. In het speltoids he occasionally found either a trivalent or an unpaired chromosome. In one of Lindhard's subcompactoids he could usually count only 41 chromosomes at anaphase, but at metaphase there sometimes appeared to be 21 bivalents. He eventually decided that there must be 42 chromosomes, of which one was usually a "vagabond" that got lost and degenerated in the cytoplasm leaving 20 to go at anaphase to one pole and 21 to the other. In

Lindhard's "squarehead heterozygote" (= "subnormal") he definitely found only 41 chromosomes. The "perennis type" also had only 41, these forming $20_{\text{II}} + 1_{\text{I}}$. A dwarf normal that segregated normals and dwarfs in a regular 3:1 ratio had the normal number of chromosomes which appeared at metaphase as 21_{II}.

Nilsson-Ehle and his associates had shown that many of the characters of wheat appear to be due to a balance between factors tending to push development in opposite directions. Winge, developing this concept further, argued that the "normal" phenotype of *T. vulgare* depends on a balance between factors on the three paired sets of chromosomes comprising its numerically hexaploid complement and that the various mutants, or "Aberranten" as he preferred to call them, are determined by changes in the balance of factors, brought about by aberrant chromosome conjugation and distribution.

If the seven pairs constituting a basic diploid set of *Triticum* chromosomes are represented as $\frac{1\,2\,3\,4\,5\,6\,7}{1\,2\,3\,4\,5\,6\,7}$, then the complement of *T. vulgare,* comprising three similar but not identical paired sets, may be represented as $\frac{1_A\,1_B\,1_C\,2_A\,2_B\,2_C}{1_A\,1_B\,1_C\,2_A\,2_B\,2_C} \ldots \frac{7_A\,7_B\,7_C}{7_A\,7_B\,7_C}$. Only one of of the triplicated pairs of chromosomes, say the $\frac{1_A\,1_B\,1_C}{1_A\,1_B\,1_C}$ group, appears to be significantly involved in the cytogenetics of speltoids and compactoids. We can therefore drop the numeral and refer to it as $\frac{A\,B\,C}{A\,B\,C}$. The C chromosomes of this group were postulated by Winge to carry factors pushing development towards the compactum type and the B chromosomes to carry factors tending towards speltoid or spelta. The normal phenotype of *T. vulgare* was assumed to be determined by the balance of factors on one pair of B and one pair of C chromosomes. Speltoids would then be produced by an excess of B chromosomes or a deficiency of C and compactoids by an excess of C or a deficiency of B. No definite function was assigned to the A chromosomes, and Winge did not distinguish *T. spelta* from speltoid.

Accepting $\frac{A\,B\,C}{A\,B\,C}$ as the relevant chromosome formula of *T. vulgare,* it is evident that chromosome A must normally pair only with

A, B with B and C with C, or true-breeding pure lines could not exist. But, Winge argued, B and C, though not identical, are sufficiently similar to pair occasionally. When bivalents AA, BC and BC $\left(\frac{\mathrm{A\,B\,B}}{\mathrm{A\,C\,C}}\right)$ or the quadrivalent BCBC $\left(\frac{\mathrm{A\,B\,B}}{\mathrm{A\,C\,C}}\right)$ are formed, gametes ABB and ACC obviously may result. If these are fertilized by normal ABC gametes the zygotes $\frac{\mathrm{A\,B\,B}}{\mathrm{A\,B\,C}}$ and $\frac{\mathrm{A\,C\,C}}{\mathrm{A\,B\,C}}$ will be produced. These would be the 42-chromosome het speltoid and the subcompactoid. The three B chromosomes of $\frac{\mathrm{A\,B\,B}}{\mathrm{A\,B\,C}}$ could then form the trivalent, and C be the unpaired "vagabond" he saw occasionally in het speltoids. He expected some het speltoids to have only 41 chromosomes, $\frac{\mathrm{A\,B\,o}}{\mathrm{A\,B\,C}}$, but did not find any of this type. The B chromosome of $\frac{\mathrm{A\,C\,C}}{\mathrm{A\,B\,C}}$ was assumed to be the "vagabond" in his subcompactoid. Because the "squarehead heterozygote" was almost normal *T. vulgare* in phenotype, though it had only 41 chromosomes, its constitution was assumed to be $\frac{\mathrm{A\quad B\quad o}}{\mathrm{A\ (BC)\ C}}$, in which the (BC) chromosome has originated by abnormal crossing-over between B and C.

On Winge's hypothesis subcompactoids should arise with the same frequency as het speltoids. They do not, and the only one of the formulae fully established today is $\frac{\mathrm{A\,B\,o}}{\mathrm{A\,B\,C}}$ which Winge expected but did not find. It is the constitution of a Series β het speltoid. Yet Winge's ideas have undoubtedly helped greatly in the elucidation of the general problem. Nilsson-Ehle (131) showed the difficulties that part-mutations create for Winge's theory, but he considered that it might explain many of the genetic complexities previously inexplicable. He recorded, however, that H. Ekstrand had examined his α, β and γ Series speltoids and found only normal chromosome numbers and behaviour in all of them.

Almost coincidently with Winge, Goulden (57) had begun cytogenetic studies of dwarf wheats and fatuoid oats, and shortly afterwards Huskins (65), at the suggestion of Dr. J. R. Fryer, had independently begun cytogenetic studies of fatuoids. Though only

Series α fatuoids had previously been known, 20 het fatuoid plants that developed from off-type seeds picked out by Goulden from supposedly pure-line Banner oats comprised three ratio-types. One produced more or less equal proportions of het fatuoids and dwarf fatuoids. The latter were the only plants he studied cytologically. They had extremely irregular pairing in pollen mother-cell meiosis, and this prevented an accurate chromosome count being made. Goulden attempted to apply Winge's concepts to the behaviour of certain dwarf wheats. Regarding the dwarf oats, he concluded that insufficient data were available for a sound explanation of their occurrence and behaviour, but suggested that "the cytological irregularities indicate that something is lacking which has a profound influence on the co-ordination of the chromosomes during reduction division. This may be either a single factor, a part of a chromosome containing several to many factors, or one or more whole chromosomes. The fact that all the dwarf plants are false wild seems to indicate that at least a part of a chromosome which also carries the cultivated factor has somehow been lost". We now know that "false wild" dwarfs of this type have only 40 chromosomes.

Huskins' (65, 66) first studies were on Series α fatuoids and het fatuoids obtained by crossing them with normal oats. Chromosome irregularities were found which suggested that "some chromosomal aberration, rather than a change in a single gene, is instrumental in causing the appearance of fatuoids". The extension of Winge's theory to fatuoids was favoured, though it was felt that "it is not clear how all the irregularities could be explained on this basis". In immediately succeeding studies, Huskins (67, 69–71) examined α, β and γ Series speltoids obtained from Nilsson-Ehle and Åkerman. He found also that a large collection of fatuoids, obtained from various sources, comprised three essentially similar Series. In 11 strains of Series α fatuoids and speltoids the normal chromosome number was found in all classes of segregates, but in het speltoids and het fatuoids a trivalent and a univalent was occasionally found. In speltoid and fatuoid segregates a quadrivalent sometimes occurred, as Winge had found in the Series α speltoids which he obtained from Lindhard. Series β het fatuoids and het speltoids were found by Huskins to have only 41 chromosomes, and these regularly formed $20_{II} + 1_{I}$ at pollen mother-cell metaphase. The chromosome constitution of γ Series strains proved very difficult to determine. Counts varying from 41 to 43 were made in both het fatuoids and het speltoids. In some fatuoid and speltoid segregates of Series γ only the normal chromosome number could be found, but in a few cells of several different plants 44 were counted. It was decided that the higher counts must be the correct ones and that technical difficulties were preventing a full count in those that appeared to have fewer than 44. The conclusion was therefore reached that the constitutions of het speltoids and het fatuoids of the α, β and γ Series were, respectively, $\frac{\mathrm{A\,B\,B}}{\mathrm{A\,B\,C}}$, $\frac{\mathrm{A\,B\,o}}{\mathrm{A\,B\,C}}$ and $\frac{\mathrm{A\,B\,C\,B}}{\mathrm{A\,B\,C}}$, while the speltoid and fatuoid segregates from them were $\frac{\mathrm{A\,B\,B}}{\mathrm{A\,B\,B}}$, $\frac{\mathrm{A\,B\,o}}{\mathrm{A\,B\,o}}$ and $\frac{\mathrm{A\,B\,C\,B}}{\mathrm{A\,B\,C\,B}}$. A part-chromosome deficiency was observed in one Series γ het speltoid, but it was not thought related directly to the speltoid problem, as it was later found to be.

These early observations of the writer, like those of Winge and Goulden, were made on paraffin-embedded and sectioned material. In them the analysis of irregular configurations and behaviour is very difficult when the chromosome number is as high as in hexaploid wheat and oats. Since 1929 his studies and those of his associates have almost all been on permanent smear preparations. In these, Series γ het speltoids and fatuoids characterized by the deficiency of part of a chromosome, rather than by an extra one, were soon found (75, 76). The same observation was independently made shortly afterwards (134, 135, 192, 193). Though counts of 43 chromosomes have been made in Series γ het speltoids by the writer as well as others (18, 58, 120), it now appears certain that these were either erroneous interpretations, or that extra chromosomes other than those affecting the speltoid complex were present. The latter is definitely the case in some of the writer's plants which have been re-examined and further analyzed. Håkansson (58) at first interpreted certain configurations as heteromorphic bivalents of which one member had lost a large segment. He later decided that they were probably trivalents composed of three small chromosomes of equal size. His first impression now appears to have been correct. The error is readily explicable: the normal C chromosome has a sub-median "attachment" constriction dividing it into two "arms"; when most of one arm is missing, as the longer one is in some γ strains, including that studied by Håkansson, the conjugation of a two-armed normal chromosome with a deficient one-armed C chromosome gives a three-partite body that may easily be mistaken for a trivalent composed of three small whole chromosomes. Müntzing (120), though he did not find the deficiency now known in the C chromosome of Series γ speltoids, stressed the probable significance of that illustrated by Huskins (71). The characteristic complement of Series γ het speltoids or het fatuoids is now known to be 20 normal bivalents and a heteromorphic bivalent composed of one normal C chromosome and one C visibly deficient at metaphase. The deficiency ranges in length from the whole of the longer arm to a segment on the limit of microscopic detectability. This segment must normally carry factors that prevent expression of the speltoid or fatuoid phenotype (76, 81, 82, 84, 111, 113, 134, 135, 152, 164, 192, 197). The chromosome number 41 found (198) in a het speltoid from Philiptschenko's (143) Series γ was evidently that of one of the Series β het speltoids which are regularly segregated by γ Series strains (82). The chromosome constitution now established for various oat and wheat mutants are shown diagrammatically in Figures 3 & 4.

Nishiyama (133) made extensive cytological studies of three Series α fatuoid strains obtained from the writer. While occasional unpaired chromosomes, trivalents and quadrivalents were found, Nishiyama concluded that the meiotic irregularities bore no relation to the plant phenotype and therefore gave no basis for the Winge-Huskins scheme of whole B and C chromosome substitutions. Uchikawa (197), from detailed cytogenetic studies of all three types of speltoids, reached the same conclusion. The total frequency of irregularities found by both Nishiyama and Uchikawa is, however, much lower in all classes of segregates than that observed in some pure varieties and many hybrids as well as in mutants of wheat and oats by many prior and subsequent workers (see 20, 95, 114, 179, 199). The Japanese workers have used paraffin-sectioned material, and it is the opinion of the writer that their observations are limited thereby, though they appear to be among the most accurate and detailed ever made in cereals by the older methods.

There is no doubt that some of the writer's (69–71) original figures of multivalent configurations in Series α were erroneous interpretations of interlocked bivalents or of bivalents with unterminalized chiasmata. Most of them, however, on re-examination are considered probably correct, and the same may be assumed for the observations of Winge, Müntzing, Håkansson and others. The question is, how significant are they for the fatuoid or speltoid problem? If Series α arises through "loss mutation", as do Series β and γ, but by a smaller minute deficiency that is not detectable microscopically by present methods, then multivalent formation in mutants of this Series is no direct indication of their constitution.

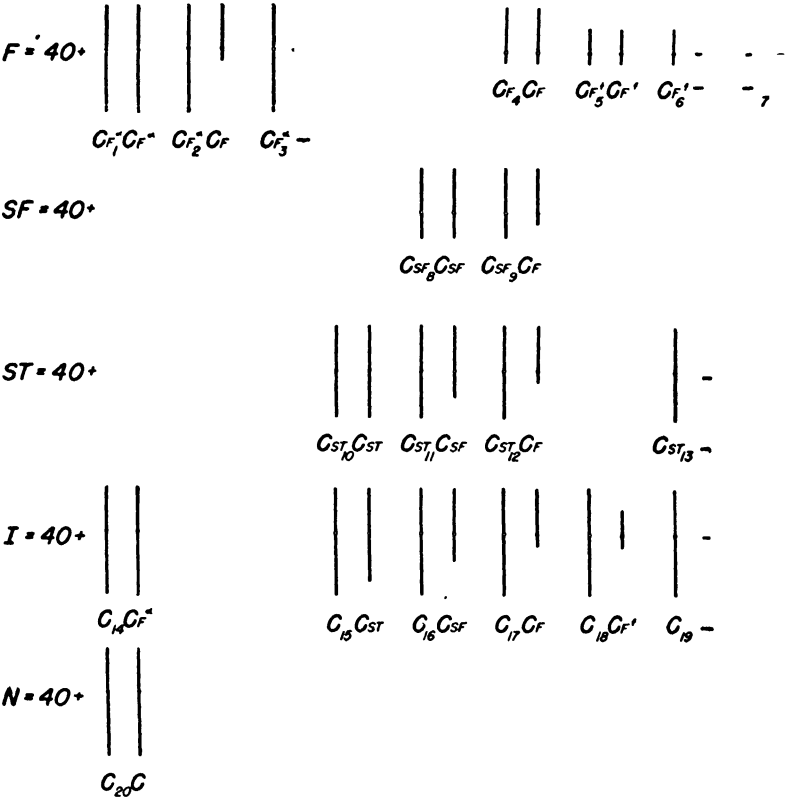

FIG. 3. Diagrammatic representation of the chromosome constitutions of fatuoids (F), subfatuoids (SF), steriloids (ST), intermediate or heterozygous mutants (I) and normal oats of *Avena sativa* L. or *A. byzantina* Koch. Normal oats in these hexaploid species have 42 chromosomes (21_{II}). The above forms are shown as having 40 chromosomes plus (or lacking) various modified forms and numbers of the "C" chromosome which carries factors that primarily determine the glume and awn characters.

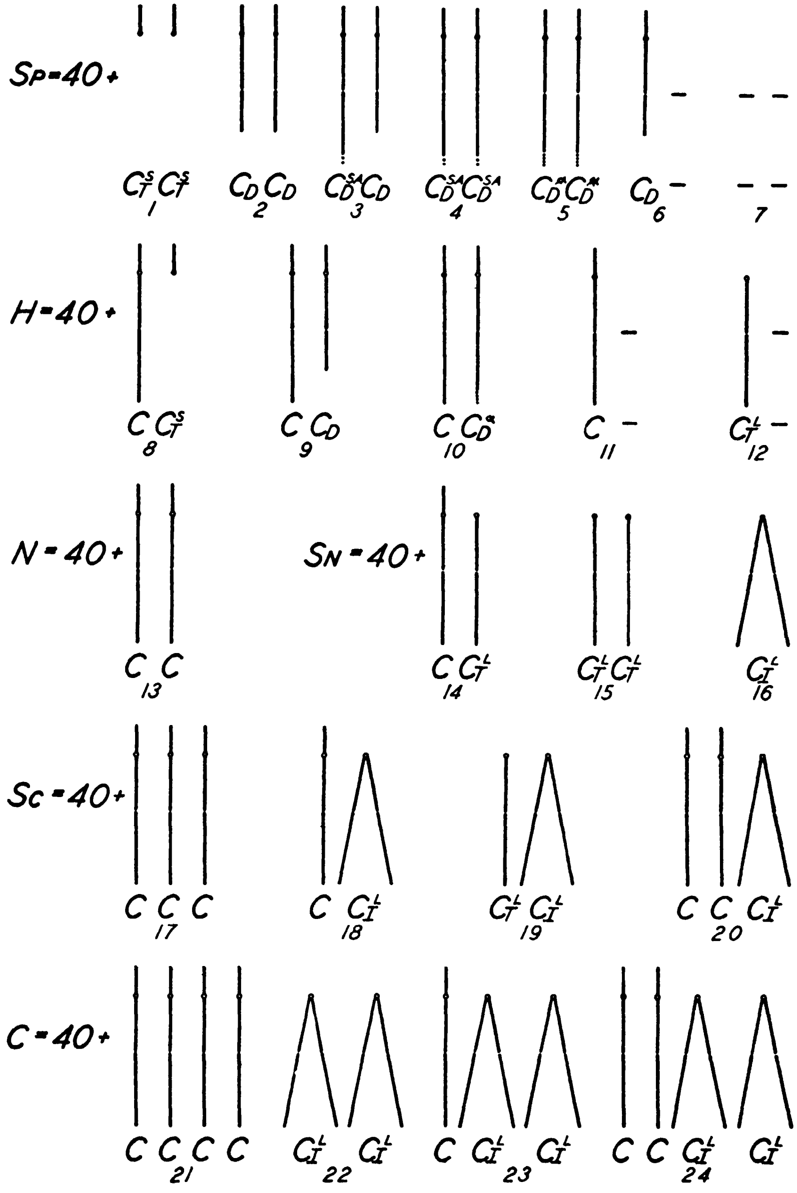

FIG. 4. Diagrammatic representation of the chromosome constitutions of speltoid (Sp), heterozygous speltoid (H), normal (N), subnormal (SN), subcompactoid (Sc) and compactoid (C) forms of common wheat. Normal wheats of any of the hexaploid species have 42 chromosomes (21_{II}). The forms above are shown as having 40 chromosomes plus (or lacking) various modified forms and numbers of the "C" chromosome which carries factors that primarily determine the normal glume, awn, and head-shape of common wheat.

It does, however, reveal the capacity for irregular pairing which exists in *T. vulgare* or *A. sativa,* and this remains one of the most likely mechanisms for the production of such chromosome mutations. The most abundant and definite evidence of multivalent formation found by Huskins (71) was in a "speltoid" strain that he suspected of being the derivative of a cross with *T. spelta,* as it has since been proved to be. It is rather ironical that the present re-analysis of Lindhard's and Winge's papers has led to the suspicion that the "speltoids" in which Winge found the multivalents, from which he formulated his theory of the origin of speltoids, were possibly also *T. spelta* hybrids (see 77). If they were not, the most plausible assumption is that extra chromosomes other than C were present in these lines. Such have been found in many of the writer's strains. In either case, frequent multivalent chromosome associations would be expected. It appears significant that Winge found no multivalents in a Series α strain that he obtained from Åkerman, and that Lindhard reported that his "speltoids" had hard and tight glumes like those of typical *T. spelta.* In a personal communication to the writer in 1927 he went further and expressed the opinion that there is no difference between speltoid and *T. spelta.* This conclusion is unacceptable (see 205), but it would come naturally to Lindhard if the vigorous segregates which he quite naturally considered to be speltoids, and from which he gave cytological material as such to Winge, were really *T. spelta* hybrids.

In any event it is apparent that these speltoids of Lindhard's on which Winge based his theory were not typical Series α mutants in either their genetic or cytological behavior, and the present general conclusion is that Series α speltoids and fatuoids are not specifically characterized by a multivalent chromosome association. But this does not mean that Winge's theory of origin through pairing of non-homologous (or, better, "homoeologous")[3] chromosomes, is necessarily invalid. Multivalents are indeed formed occasionally in all oats and wheat whether mutant or normal, and Watkins (206) and others have shown genetically that autosyndesis, which is the equivalent of the B + C pairing postulated by Winge, occurs regu-

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[3] Homoeologous chromosomes (73) are those which are phylogenetically alike in a polyploid species. They once were homologous or identical but are now only similar, being sufficiently alike to synapse occasionally and so to form multivalent associations but not to pair regularly—homology though essential for regular pairing and crossing over does not, of course, in itself ensure pairing!

larly in certain tetraploid hybrids. In 0.3% of Uchikawa's (197) 41-chromosome Series β het speltoids a trivalent was formed and no univalents were present. In this material the C chromosome must have been paired with a non-homologous or homoeologous pair, though the significance of this appears to have been overlooked by Uchikawa. When Winge presented his hypothesis the chromosome was commonly considered a more unitary structure with respect to pairing than it is now known to be. We now stress synapsis between segments of chromosomes, but the associations formed by any segment affect the pairing possibilities of its neighbours. The "pairing block" (35), a dynamic entity, is now generally considered the functional unit.

Series γ fatuoids and speltoids are undoubtedly due to genetic deficiency, determined by the various segmental chromosome losses seen in different strains of Series γ, and there is every gradation between the loss of a whole C chromosome in Series β to the minute deficiencies found in some Series α strains. In most Series α strains, however, no deficiency can be observed in the chromosomes at metaphase, and the question then arises whether we are dealing with a gene mutation affecting the epistatic factors of the C chromosome or with the loss of them. For this we turn to genetic evidence.

The argument of Nishiyama (135), Uchikawa (197) and of Jones in his earlier work (89) that Series α must be due to gene mutation and not chromosome aberration (as are Series β and γ) can not be held as a necessary conclusion from their extensive genetic and cytological observations. The speltoid mutation involves at least two genes, or, more probably, two complexes of genes—those affecting glume and awn characters. In addition, there is between these two genes or gene complexes, an interstitial region, 30–40 crossover units long, which must be involved in the mutation. Were it not, part-mutants would regularly arise from het speltoids through crossing over in this region. The general concept of gene mutation certainly does not embrace such complex changes as those here involved, and Uchikawa's conclusion that Series α speltoids arise by gene mutation is therefore quite unacceptable. For fatuoids, however, the gene mutation theory can not as yet definitely be discarded. The fatuoid complex of characters is determined by either one gene or one complex of genes within which no crossing over has been established. The subfatuoid and steriloid mutations are not yet proved to be changes necessarily involving different loci from the fatuoid mutation; they could, on present evidence, be multiple alleles, that is, different changes at a single locus. They have, however, at least been accompanied by different visible changes in the C chromosome in all the cases so far studied by the writer and his associates, and this together with all the other accumulated evidence makes chromosome mutation, such as deficiency, a far more plausible explanation of their origin than gene mutation.

If simple deficiencies are involved, as suggested (9, 76, 91, 120), these could owe their origin to the presence of a duplicated segment in the normal C chromosome. Huskins and Smith (83) have found that the single C chromosome in Series β het speltoids sometimes pairs on itself. This is evidence of segmental duplication within the C chromosome, and it provides a possible mechanism for the origin of deficiencies through irregularities in synapsis and crossing over. Pairing between the C chromosome and one of its homoeologues A or B would also produce deficiencies. The single factor ratios obtained from crossing the cultivated types with either the "wild" or the mutant forms show that only one of the three homoeologues (C) bears the epistatic "suppressors" that determine the cultivated phenotype. Crossing over during homoeologous association could therefore produce a C chromosome lacking this segment but otherwise relatively unchanged. Homoeologous pairing as the basis for the mechanism which directly produces the mutants would fit more of the data than simple segmental duplication, but convincing direct evidence for it would be harder to obtain. Both may well be involved. Efforts to settle these questions from studies of the chromosomes at the pachytene stage, when they are longer and more distinguishable, have met with only limited success. The chromosomes are too numerous and too long. In a few pachytene preparations they are relatively much shorter and clearer and from these some evidence has been obtained (81, 82).

The chromosome numbers 41 and 40 of Huskins (69, 71) for heterozygous and "homozygous" mutants of Series β have been confirmed for speltoids (58, 59, 117, 120, 147, 164, 165, 197) and for fatuoids (133). The genetic ratios of Nishiyama's 41-chromosome het fatuoids, like those of Goulden (57) and of the strains described elsewhere (80) and herein as "modified Series β" ("β mod."), differ, however, from those of ordinary Series β het speltoids or fatuoids, though they are cytologically similar. They produce few normals, many 41-chromosome het fatuoids and many 40-chromosome dwarf sterile fatuoids.

From counts of chromosomes left out of pollen tetrad nuclei, it is possible in Series β to determine the proportion of gametes with 20 or 21 chromosomes (71). Nishiyama (133) calculated that in his 41-chromosome modified β het fatuoids it was 1:6. He assumes that: (*a*) female gametes are produced in the same ratio, (*b*) that all gametes function in the proportions they are formed, and (*c*) that the relatively low fertility of these het fatuoids is due to the death of many 40-chromosome fatuoid embryos. On these assumptions good agreement is reached between expectation and the actual genetic ratios. But the explanation seems an over-simplification (Sander, 152). It is, however, clear that in modified Series β the 20-chromosome gametes function readily, whereas in ordinary Series β het fatuoids, and Series β het speltoids also, the 20-chromosome pollen grains have a very low functioning capacity in competition with normal grains (164, 165, 197).

Nishiyama confirmed the observation of others (57, 69, 70, 79) that absence of both C chromosomes causes faulty meiotic pairing, and he attempted to locate on C the factors necessary for normal pairing. The analysis was based on fertile, tall fatuoids, having normal chromosome pairing, which are found occasionally in the progeny of modified β het fatuoids. These fatuoids have $20_{II} + 1_{I}$ instead of the usual 40_{I} of Series β or β mod. fatuoids. The univalent chromosome has a sub-median "attachment". It is smaller than the normal C chromosome and was proved by its behaviour in crosses with normals to be part of it. Nishiyama (135) believes that this univalent is the longer arm of a C chromosome and that the normal C therefore bears a factor or factors necessary for synapsis on its long arm and factors that suppress the fatuoid characters on its shorter arm. This interpretation involves, however, the formation of a new spindle attachment in a chromosome fragment and also other assumptions that do not appear to be valid (152). There is, however, complete agreement among the various workers that the essential cytological characteristic of both Series β and modified β is the loss of a C chromosome. The problems remaining are the location of various factors on it and the cause of the difference between Series β and modified β in the functioning of their 20-chromosome male gametes.

Subcompactoid wheats from Nilsson-Ehle and Åkerman were studied cytologically by Håkansson (58, 60). He found two chromosome types. The less frequent type has an extra C chromosome, its formula being given as $20_{II} + 1_{III}$ or $\frac{\text{A B C C}}{\text{A B C}}$ $\left(\text{and that of its compactoid segregates as } \frac{\text{A B C C}}{\text{A B C C}}\right)$. The commoner type has $20_{II} + \text{C} + \text{Co}$, or $\frac{\text{A B C}}{\text{A B Co}}$, $\left(\text{with its compactoids } \frac{\text{A B Co}}{\text{A B Co}}\right)$, in which "Co" is a "compactoid producing" chromosome with duplicated ends. It was observed by S. G. Smith and the writer (76) and later others (95, 164, 194). As it is known now to be an "isochromosome" composed of a duplicated longer arm of the C chromosome, it is designated Cil.

When not paired with the normal C chromosome (called "No" by Håkansson) the duplicated arms of Cil pair and form chiasmata. and it therefore looks like a small bivalent. When C and Cil are paired they form a heteromorphic "bivalent," and at anaphase go to opposite poles forming gametes with 20 + C and 20 + Cil chromosomes. When unpaired, either C or Cil or both may be left out of the spore nuclei and gametes with only 20 chromosomes thus be formed. These fertilized by normal gametes produce the β het speltoids which are regular segregates from C Cil subcompactoids. Håkansson suggested that plants with 20_{II} + Cil must also occur and expected that these would be intermediate between compactoid and normal, *i.e.*, the "squarehead heterozygotes" of Lindhard, or "subnormals" as they are termed herein. This has been confirmed (83, 164, 165, 194–196). Huskins and Smith (83) have also found C C Cil subcompactoids which were expected but not found by Håkansson, and have confirmed his observation of Cil Cil compactoids. They have found, in addition, an unbalanced C Cil Cil compactoid. Håkansson noted that C Cil subcompactoids from Nilsson-Ehle had a shorter C chromosome than those from Åkerman. It appears to be the same as the "Ctl" chromosome of Huskins and Smith (83) which is the "telokinetic" or terminally attached long arm of C, which equals one-half of a Cil chromosome. The het speltoid progeny from subcompactoids of this type possess

20_{II} + Ctl, and consequently differ from ordinary Series β het speltoids in segregating subnormals (20_{II} + Ctl Ctl) instead of true normals (20_{II} + C C).

The steriloid and subfatuoid mutations may provisionally be considered part-mutations of the fatuoid complex. The same chromosome, C, is affected, and they behave like a multiple allelomorphic series with fatuoid, yet are evidently due to or at least associated with chromosome aberrations and may therefore involve different parts of a gene complex. Different sized deficiencies are involved in some cases and there are different ratio-types, as in fatuoids (80).

The relation of these various wheat and oat mutants to similar forms which segregate from interspecific hybrids has been investigated extensively (*e.g.*, 91, 104, 136, 146, 205, 206). From data on chromosome numbers and behavior, chromosome mutations and the genetics of interspecific crosses, many attempts have been made to reconstruct the phylogeny of the genera *Triticum* and *Avena* (see 46, 48, 56, 97, 115, 156, 181, 200, 205). The more detailed cytogenetic data now available on the various mutants may throw further light on these problems.

E. GENERAL CONCLUSIONS

Many off-types found in cultivated varieties of wheat and oats are definitely mutants, while others are the product of natural crosses. The types commonly described as false wild oats or fatuoids are mutants. The thesis that they arise through natural crossing between *A. sativa* and *A. fatua* is not maintained by any of the data marshalled in its support when these are critically examined. On the other hand, it is clear that some off-types which are almost indistinguishable from true mutant fatuoids, are natural hybrids or their descendants.

Speltoid wheats arise by mutation from *T. vulgare,* but, again, forms possessing either the same or a very similar complex of glume and spike characters arise as irregular segregates from various intervarietal and interspecific crosses between hexaploids or as regular segregates from the pentaploid hybrid *T. vulgare* × *T. turgidum.* The definitions of fatuoids and speltoids emphasize that they resemble the parent variety excepting in the one complex of characters and (occasionally) others genetically and developmentally associated with it. The type of mutation involved is definitely chromosome mutation in most off-type wheats and oats studied. The diverse chromosome aberrations producing different ratio types are alike in that they all involve the loss of genes that ordinarily prevent the expression of the fatuoid or speltoid characters.

Pairing between "homoeologous" chromosomes of different sets or genomes in allopolyploid or amphidiploid species and the consequent establishment of new chromosome and genic balances was the basis of Winge's and Huskins' earlier theories of the origin of various wheat and oat mutants. This surely occurs, as evidenced genetically by the occurrence of "shift" and allied phenomena in polyploid wheat hybrids. But whereas the earlier development of the theory emphasized whole chromosome substitution as the result of homoeologous pairing, it now appears that much more emphasis should be laid on the secondary effect of irregular pairing in producing segmentally altered chromosomes and also on the fact that polyploidy permits the survival of chromosomally aberrant or segmentally deficient or duplicated types of gametes and zygotes that could not survive in dipolids.

Most of the striking mutations of wheat and oats appear to be caused by whole or part deficiencies or duplications of one particular chromosome, designated C. On this C chromosome there are factors affecting most of the characters which distinguish the different hexaploid species of each genus. The C chromosomes carry also colour and disease-resistance factors in some strains, and their absence affects the growth habit of the plant and in oats causes meiosis to be disrupted. They profoundly affect vigor and fertility. Albinism and a leaf-width mutation in oats (144, 146), a white chaff mutation in wheat (112) and hairy stem in wheat-rye hybrids (95) have all been shown to be associated with aberrations of particular chromosomes other than C. By contrast there are, in both oats and wheat, chromosomes which can be lost without causing any apparent effect beyond a slight reduction in size and fertility. Sears (160–162) found that most but not all chromosomally aberrant wheats obtained from a "haploid" *T. vulgare* were morphologically distinguishable from the normal,[4] and O'Mara (140) found that of three extra rye chromosomes present in an amphidiploid wheat-rye hybrid backcrossed to wheat, two produced definite effects and the third apparently none. Irregularities in the number and behaviour of relatively non-effective or "extraneous" chromosomes are common in many mutants of the speltoid and fatuoid series. They are responsible for some of the difficulty experienced by various workers attempting to analyze these problems.

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[4] In an elegant analysis which has appeared since this manuscript was prepared (Genetics 29: 232–246. 1944), Sears has shown the contributions made by 17 of the 21 pairs of chromosomes to the phenotype of *T. vulgare* var. Chinese Spring. When both members of the pair are absent all 17 chromosomes analyzed cause appreciable deviation from the normal, but, provided the plants are grown under favorable conditions, only two chromo-

Apart from the immediate problems of the origin of speltoid, fatuoid and related mutants and their diverse genetic behavior, which are reviewed herein, the cytogenetic analysis of these forms has given data which bear on problems of chromosome mechanics, especially in polyploids, on pseudo-allelism and the concentration of genes on a single chromosome, on the significance of polyploidy in relation to mutation, the pure-line theory and plant-breeding practices, and on the evolutionary history of *Triticum* and *Avena*. These problems, merely outlined above, are considered in some detail elsewhere (81, 83).

F. SUMMARY

Fatuoids, speltoids and related oat and wheat mutants arise through chromosome aberration, and their diverse genetic ratiotypes are determined by their particular chromosome constitutions. These are shown diagrammatically in Figures 3 and 4.

(*a*) Loss of one C chromosome produces a Series β het fatuoid or speltoid. The remaining C chromosome, being univalent at meiosis, "splits" after the members of the bivalents have separated to the poles, but its halves often fail to be included in the daughter nuclei. The gametes therefore possess either 20 or 20 + C chromosomes, with a marked preponderance of the former. Female gametes of either constitution function readily, but male gametes lacking C rarely effect fertilization. The characteristic ratio of Series β, which is one normal to more than four heterozygotes (the number differs somewhat in different strains) is thus determined. Rarely, Series β het speltoids have 20_{II} and a C chromosome lacking the shorter arm ("Ctl" since it is the telomitic longer arm of C); they segregate subnormals (20_{II} + Ctl Ctl) instead of true normals.

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somes show appreciable effects when only one member of the pair is lacking. One of these two is the C chromosome, which he designates number IX. He confirms the earlier observations, reviewed herein, that it carries factors for squareheadedness, narrow glumes and suppression of awns, and shows further that chromosome X also carries an awn suppressor, while factors for the promotion of awn development occur on chromosomes II and XX.

(*b*) Modified Series β strains have chromosome number and behavior the same as in typical Series β. Both female and male gametes lacking a C chromosome are, however, functional in these strains; many sterile, dwarf, 40-chromosome fatuoid progeny are therefore produced by modified β het fatuoids.

(*c*) Loss of an appreciable part of one of the C chromosomes produces Series γ het speltoids or het fatuoids; their formula is $20_{II} + C\ Cd$ (Cd = a chromosome having an appreciable deficiency). The size of the deficiency varies in different strains, and there may be a correlation between it and the genetic ratio. In most strains the C and Cd chromosomes pair and disjoin fairly regularly, and gametes with 20 + C and 20 + Cd are therefore formed in approximately equal numbers. Ovules of both types function readily, but pollen grains with 20 + Cd effect fertilization only rarely—hence the production of normal and het fatuoid progeny in ratios approximating 1 : 1. The few 20 + Cd male gametes which function and fertilize similar female gametes produce the few Cd Cd speltoid or fatuoid progeny. These are more or less dwarf and sterile. When the C and Cd fail to pair and behave as univalents, their halves frequently fail to enter the daughter nuclei. Gametes are therefore formed with only 20 chromosomes, and these fertilized by normal, 20 + C, gametes form the $20_{II} + C$, Series β, heterozygotes that occur with appreciable frequency in the offspring of Series γ het fatuoids or speltoids. A 20-chromosome gamete (lacking C) fertilized by a 20 + Cd gamete gives a 41-chromosome speltoid or fatuoid. In one γ speltoid strain the deficiency involves the whole of the longer arm of the C chromosome; the formula for the het speltoid is then $20_{II} + C + Cts$ (Cts = telomitic short arm of C).

(*d*) Series α het fatuoids or speltoids segregate as if they differed from the normal by a single unit factor, and it has therefore been argued that they arise by gene mutation. Few strains of fatuoids and almost none of speltoids, however, give good 1 : 2 : 1 ratios. There is nearly always a deficiency of the homozygous mutant progeny, and sometimes the ratio may be nearer 1 : 1 : 0 than 1 : 2 : 1. It is then a matter of definition whether they be classed as Series α or γ. Those giving ratios closely approaching 1 : 2 : 1 have no chromosome deficiency large enough to be detectable at meiotic metaphase. Some het speltoids which are intermediate and have by Nilsson-Ehle and others been classed as Series α have a visible deficiency. The minimum deficiency involved is probably greater in the speltoid total-mutation, since it involves two genetically separable genes or gene complexes, than in the fatuoid, which is apparently determined by one gene complex. The deficiencies observed could have arisen either through intrachromosome pairing of a duplicated segment which has been found in the C chromosome or through pairing between C and some other phylogenetically related chromosome. If caused by such "homoeologous" pairing, the deficiency may arise *de novo* or may be a transference to the C chromosome of a phylogenetically old relative-deficiency. The occurrence of "speltoids" as regular segregates from interspecific wheat hybrids bears on the latter point and also constitutes evidence, of an *a priori* nature, that whole chromosome substitution as first envisaged by Winge may possibly be one cause of true speltoid mutations, though the existence of such substitution speltoids is not yet definitely established. There is, however, no unequivocal evidence for one specific "B" chromosome carrying a complex of factors hypostatic to those of C and thus none for the original formulae of the Winge-Huskins hypothesis. The general basis of this hypothesis appears sound, but its detailed development has been greatly modified. The "speltoids" of hybrid origin and, even more, the *A. sativa* × *A. fatua* segregates often mistaken for fatuoids, must be considered as posing distinct problems to be related in a phylogenetic analysis, but not to be confused with mutants in any investigation of either.

(*e*) Steriloid and subfatuoid mutations involve deficiencies of different sizes in the C chromosome of *A. sativa.* Normal, steriloid, subfatuoid and fatuoid behave, however, as if they were due to descending members of a series of multiple alleles. The C chromosome carrying the "steriloid deficiency" occurs as a univalent in a modified β strain of steriloids. Segregating steriloids of this type are phenotypically identical with true-breeding steriloids. They have 41 chromosomes and give rise to a few 42-chromosome true-breeding steriloids, many segregating-steriloids and 40-chromosome dwarf fatuoids which lack the normal synaptic association of meiosis.

(*f*) Subcompactoids of the commonest type (Type 1) have a "Cil" chromosome; it is an "isochromosome" composed of a duplicated longer arm of the C. Their total complement is $20_{\text{II}} + \text{C} + \text{Cil}$. Rarely, similar forms are segregated with $20_{\text{II}} + \text{Ctl} + \text{Cil}$.

(*g*) "Subnormal" mutants have a Cil chromosome but lack a normal C. More rarely they have two separate Ctl chromosomes in place of a Cil chromosome (which is two united longer arms).

(*h*) A second type of subcompactoid is a "primary trisomic" having three C chromosomes—20_{II} + C C C.

(*i*) A third type of subcompactoid has 20_{II} + C C + Cil.

(*j*) Common types of compactoids have 20_{II} + Cil Cil, 20_{II} + C C C C, or 20_{II} + C + Cil Cil.

ACKNOWLEDGMENTS

I am indebted to Dr. H. G. Sander for much assistance in the reviewing of literature and the critical revision of this paper.

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THE BOTANICAL REVIEW

VOL. XII NOVEMBER, 1946 No. 9

CYTOPLASMIC INCLUSIONS OF THE PLANT-LIKE FLAGELLATES. II[1]

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During the past ten years, investigations on the plant-like flagellates have included a number of papers on Golgi material and on various types of subcuticular bodies, the latter a relatively neglected group of inclusions. The vacuome and the mitochondria have received less attention, although both types of inclusions have been described in certain species.

GOLGI MATERIAL

The identity of flagellate Golgi material remains somewhat uncertain, since different types of inclusions have been recognized as "Golgi apparatus" in closely related and even in identical species. Recent papers have identified the Golgi material as scattered dictyosomes (14–16), as an osmiophilic cortex of the contractile vacuole (9–11), as osmiophilic bodies applied to the base of the gullet (reservoir) in Euglenida (9), and as osmiophilic globules adherent to the contractile vacuole (10).

Criteria for the recognition of flagellate Golgi apparatus have remained more or less flexible, and the ultracentrifuge (18) has afforded no critical evidence in identification. Positive osmic impregnation has remained the favored criterion. On the other hand, "dictyosomes" have been reported, without successful impregnation, in material stained with iron hematoxylin after osmic fixation (14). In some instances, at least, silver impregnation has proven unsatisfactory (9). Gatenby and his associates have contended that any inclusions which react to vital dyes (*e.g.*, neutral red, methylene blue) can not be considered Golgi material. However, the soundness of this position now seems a bit uncertain in view of Worley's

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[1] Supplement to article in The Botanical Review 2: 85–94. 1936.

(19) apparent demonstration that Golgi material in various invertebrates and vertebrates can be stained supravitally.

The dictyosome-type of Golgi apparatus has been described in *Distigma proteus* (14) and also in certain other Euglenida (15)—species of *Anisonema, Entosiphon, Heteronema, Parastasia, Peranema* and *Petalomonas.* The form of these dictyosomes, constant for a given species, varies from one species to another, although each dictyosome appears double, as if in division (15). Such Golgi bodies are osmiophilic (15), are demonstrable also in hematoxylin preparations (14), are not stainable with vital dyes, and are considered distinct from the vacuome. The number of dictyosomes varies widely with the species—only a single body in *Parastasia grassei* (15), three in *Distigma proteus* (14) and more than 25 in *Heteronema acus, H. mutabile* and *Peranema trichophorum* (15). However, such dictyosomes were not demonstrated in *Euglena* sp. (9, 18) or in *E. viridis* (10). Whether these inclusions are comparable to the previously described "Golgi bodies" of *Euglena gracilis* (1, 2) is uncertain. Similar discrete Golgi bodies have been described in several Phytomonadida—*Eudorina illinoisensis, Pandorina morum* and *Volvox globator* (16)—as dictyosomes which are larger than elements of the vacuome and often appearing as perinuclear crescents with their concavities toward the nucleus.

Golgi material of different types has been described in other flagellates. That of *Chilomonas paramecium* (Cryptomonadida) has been identified as the cortex of the contractile vacuole (11), an interpretation proposed previously by Nassonov. In fission, the osmiophilic cortex is said to become separated from the contractile vacuole and subsequently divided between the daughter flagellates (11). Patten and Beams (18), on the other hand, failed to note impregnation of the contractile vacuole in this species.

The Golgi material in *Copromonas subtilis* (Euglenida) may appear as an osmiophilic cortex of the reservoir, or as an osmiophilic vesicle or a group of osmiophilic granules in contact with a non-osmiophilic reservoir (9). An apparent division of the osmiophilic material, after separation from the reservoir, also has been described. Gatenby and Singh (9) concluded that the reservoir may collapse, disappear completely, and then be replaced by a growing osmiophilic vacuole, the wall of which represents Golgi material. This interpretation is unique, since the reservoir of Euglenida usually seems to be a permanent structure in flagellated stages and even undergoes enlargement and division during binary fission.

Patten and Beams (18) were unable to reach a definite conclusion concerning the identity of Golgi material in *Euglena* sp. However, Gatenby and Singh (9), after studying the preparations of Patten and Beams, identified the Golgi apparatus of this flagellate as numerous "loaf-shaped" osmiophilic bodies applied to the wall of the "contractile vacuole". Actually the Golgi material figured by these workers appears to lie on the wall of the reservoir, and it is likely that their "large bladder-like structure" in contact with the "contractile vacuole" (9) was merely the contractile vacuole in contact with the reservoir. The Golgi material of *Euglena gracilis* has been described as the osmiophilic cortex of the reservoir, and that of *Euglena viridis* as osmiophilic bodies applied to the wall of the contractile vacuole (10).

VACUOME

In the ultracentrifuge technique (18) elements of the vacuome are stratified with the carbohydrate reserves in *Chilomonas paramecium,* with the paramylum bodies in *Menoidium* sp., and pass toward the centrifugal pole in *Euglena* sp. Since Golgi material was not identified in these flagellates, there was no comparison of vacuome with Golgi material. However, Patten and Beams (18) did not believe that the vacuome should be considered Golgi material.

Positive tests for volutin have been reported for the vacuome in *Chilomonas paramecium* (11, 18), *Copromonas subtilis* (9), *Euglena* sp. (18), *E. pseudoviridis* (5), *E. gracilis* var. *urophora* (8) and *Peranema trichophorum* (6). These observations support the conclusion of Baker (1) and earlier workers.

Brief descriptions of the vacuome have been published for *Distigma proteus* (14), *Entosiphon* sp. (6), *Euglena archaeoplastidiata* (5), *E. gracilis* var. *urophora* (8), *E. pseudoviridis* (5), *E. stellata* (7) and *Scytomonas pusilla* (6). In addition to the typical vacuome of *E. pseudoviridis,* Chadefaud (5) has described a "periflagellar vacuome"—certain swellings of the flagellum anterior to the stigma. Each swelling contains an elongated mass which stains like the vacuome. The vacuome of *E. archaeoplastidiata* is unusual in that the majority of the inclusions lie between the periplast and the chromatophores, mainly in the equatorial region (5). Some of the vacuoles are larger than the elements of the vacuome usually seen in Englenida, others appear as aggregates of small granules, and some even form branching filaments which may anastomose.

As noted previously (13), various workers have suggested the possible identity of vacuome and Golgi material. This suggestion has met opposition. For example, Gatenby, Singh and Browne (10) find no reason for homologizing these inclusions with Golgi material, although they point out that the vacuome "may stain deeply in osmium tetroxide, and may resist decoloration even longer than the Golgi apparatus (osmiophile material associated with the flagellum vacuole complex)". However, Worley's (19) observations, which indicate that metazoan Golgi bodies are frequently stainable with vital dyes, may have reopened this question of the relation between "vacuome" and "Golgi apparatus".

MITOCHONDRIA

Recent work on mitochondria of flagellates includes the description of small granular mitochondria in *Distigma proteus* (14); granular mitochondria, often aggregated around the nucleus, in *Chilomonas paramecium* (11); rod-like mitochondria, demonstrable by both silver and osmic Golgi techniques, in *Copromonas subtilis* (9); peripheral mitochondria in *Peranema trichophorum* and *Entosiphon sulcatum* (6). In the ultracentrifuge method (18) the mitochondria of *Euglena* sp., in contrast to elements of the vacuome, have shown centripetal stratification.

SUBCUTICULAR INCLUSIONS

Subcuticular bodies, apparently distinct from vacuome and mitochondria, have been described in a number of species. The inclusions may be globular, as in *Euglena archaeoplastidiata* (5), or they may be fusiform rods, as in *Gonyostomum semen* (3, 4), *Euglena amphipyrenica* (5), *E. stellata* (7), *Lepocinclis radiata* (5) and *Peranema trichophorum* (6). The fusiform rods lie perpendicular to the surface in some species, but more or less parallel to the periplast in others. The inclusions may be aligned beneath the cuticular striations, as in *L. radiata* and *E. archaeoplastidiata* (5). In some cases they seem to be attached through the periplast to the striations and may be carried away with the periplast in plasmolysis of the flagellate (5). Similarly, the "mucus-vesicles" of *Euglena intermedia* (12) seemed to be joined by canals to excretory pores in the periplast.

On the basis of staining reactions, two general types of these inclusions have been recognized (6): iodophilic bodies not stainable with cresyl blue or neutral red, and non-iodophilic inclusions which are stainable with vital dyes. Subcuticular bodies reacting to vacuome stains have been described in *Euglena gracilis* var. *urophora* (8), *E. stellata* (7), *E. viridis* (3) and *Lepocinclis radiata* (5). Iodophilic inclusions have been reported in *Distigma proteus* (14), *Entosiphon* sp. (6), *Euglena archaeoplastidiata* (5) and *Peranema trichophorum* (6). In some instances these inclusions are preserved by mitochondrial fixatives but are destroyed by treatment with dilute acetic acid (5).

According to one viewpoint, these subcuticular bodies of flagellates are to be considered secretory globules stored in the cortex—the "mucus-globules" and "mucus-vesicles" of Grassé and Poisson (12) and earlier workers. Another interpretation is that such inclusions are trichocysts analogous to those of ciliates (3, 5, 6, 11, 17).

In some cases, detailed observations tend to support the latter interpretation. Thus, in *Chilomonas paramecium* (17) the subcuticular bodies are said to be discharged as long threads under certain conditions. In fixed and stained preparations of the same species, threads adherent to the periplast have been interpreted as filaments extruded from the small subcuticular "trichocysts" (11). Comparable trichocysts have been reported in several species of *Ceratium* and also in *Diplopsalis lenticulata, Peridinium tripos* and *Polykrikos Schwartzi* (17). Two varieties of trichocysts have been described in the chloromonad, *Gonyostomum semen* (3, 4)—fusiform trichocysts lying perpendicular to the periplast, and small granular ones, both types showing general distribution in the cortex. Both types of trichocysts are discharged to produce long filaments when the flagellates are treated with cresyl blue. The "trichocysts" of various other flagellates—*e.g.*, *Euglena archaeoplastidiata* (7), *Peranema trichophorum* (6)—do not give rise to filaments, although expulsion of the contents may occur after treatment of the flagellates with solutions of iodin or cresyl blue. The expulsion of discrete "globules" has been reported in *Euglena stellata* (7) and *P. trichophorum* (6). Such "trichocysts" might conceivably be analogous to the fluid-filled trichocysts of certain ciliates.

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PLANT TISSUE CULTURES. II[1]

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The idea of cultivating excised tissues as a means of studying intercellular and intraorganismal correlations was first clearly formulated in 1902, in a now classic paper by Haberlandt (35). Since that time the field of plant tissue cultures has been reviewed many times (14–16, 39–41, 77, 78, 81, 83, 86–88). The most recent and comprehensive treatment is White's "Handbook of Plant Tissue Culture", published in 1943 (89). The present paper, written as a decennial supplementary literature survey, is intended to bring the subject as nearly as possible up to date, especially to cover in detail the years 1942, 1943 and 1944.

Plant tissue cultures may be divided into four main classes, according to the particular tissues or organs involved: *a*) root cultures, *b*) embryo cultures, *c*) cultures of other members such as stem tips, and *d*) cultures of undifferentiated masses. During the developmental phases of the subject, while methods, nutrients, *etc.*, were matters of prime importance, the first of these classes was, for technical reasons, much more intensively studied than any other (81, 88). This is still true. Roots of a dicotyledonous tree, *Acacia melanoxylon,* have been cultivated for the first time (1). The observed increment rates (*ca.* 1.0 mm. per day) were very much less than those recorded for most herbaceous plants (5.0–20.0 mm. per day). This slow growth rate may be characteristic of trees. There is also the possibility that the experimental methods and nutrients, developed for roots of herbaceous plants, may not be entirely satisfactory for woody plants. These questions remain unanswered. Roots of a monocotyledonous plant, *Zea mays,* have likewise been cultivated for extended periods for the first time (47). Five per cent glucose and an agar substratum were used as compared with the 2% sucrose and liquid medium which seems to be superior for roots of dicotyledonous plants. Robbins has demonstrated a specific morphogenetic response of *Datura* root hairs to light, *in vitro* (68). Bonner has demonstrated an inhibitory effect, reversible by application of *p*-amino-benzoic acid, resulting from treatment of tomato roots with sulfanilamide, sulfapyridine and sulfathiazole (3).

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[1] Supplement to article in The Botanical Review **2**: 419–437. 1936.

Robbins found β(-4-methylthiazolyl-5)-alanine capable of substituting for thiamin-thiazole in the nutrition of tomato roots (67). He also showed pyridoxine to be highly specific in its effects in the nutrition of tomato roots (70). Tomato roots were observed to respond to nutrition with thiamin plus pyridoxine but not to thiamin plus glycine, while different strains responded differently to niacin (4). Similar results were obtained elsewhere (11, 12). Roots of pea and of oats were shown to be inhibited by indole-acetic acid at concentrations of 10^{-6} M or more (5).

Although these papers still indicate a considerable interest in method, the major emphasis has shifted from how to grow roots to what can be done with roots when grown. Henderson and Stauffer used excised tomato roots in a study of respiratory processes (38). Thiamin synthesis by maize roots has been demonstrated (47), and synthesis of ascorbic acid by excised tomato roots studied (66). Bonner demonstrated synthesis of thiamin, niacin and riboflavin by roots of a number of species and subspecific strains of plants (2, 4). Dawson has employed the technique in a particularly interesting way as final proof of the localizations of synthesis of nicotine and other alkaloids in roots of tobacco (10), originally demonstrated by means of reciprocal grafts. Hartt has used a similar technique in studying the syntheses and interconversions of sugars in roots of sugar cane (37). The problem of heterotic vigor has likewise been investigated by means of excised root tips (69, 80), while others studied the effects of polyploidy on growth rates of excised tomato roots (71).

The behavior of excised stem tips of sunflower under the influence of a great variety of growth substances has been examined (74). Most substances which promoted rooting or tended to improve the condition of roots when formed showed favorable effects on stem-tip growth, but since these effects generally became evident only after roots were present they were probably indirect in nature. Loo (44) has made a somewhat more clear-cut contribution in cultivating excised stem tips of asparagus *in vitro* for nine months and 20 transfers, studying their organic nutrition.

Although the cultivation of excised embryos is not, strictly speaking, tissue culture, it is so closely related to the cultivation of tissues and has received such a stimulus from tissue culture studies (*vide* the 1944 Conference on Plant Embryo Culture at

Smith College) as to justify its inclusion here. This subject had been touched on cursorily many times in the past four decades (13, 36, 42, 43, 65, 75, 76, 79, 82). Recently there has been a marked increase in such studies. Van Overbeek, Conklin and Blakeslee demonstrated the effectiveness of certain constituents of coconut milk in the nutrition of excised embryos of *Datura* (58, 59), and others made preliminary studies of the chemistry of these constituents (60). Development of embryos in culture, without special reference to their nutrition, has been investigated in *Hordeum* (48). Work with excised embryos of *Iris* (64) and with cabbage (9) has indicated that the seed coat contains an inhibitor which retards germination of the seed. Gregory and his associates (33, 34, 62, 63, 72) studied the vernalisation of excised embryos and parts of embryos of rye, thus segregating the factors affecting the embryo directly from those acting through the endosperm. Loo and Wang have likewise cultivated embryos of conifers (45). The method is beginning to have considerable importance in the study of interspecific hybrids where embryo development is defective for nutritional reasons.

The last class of materials for cultivation, undifferentiated or only slightly differentiated tissue masses, is of especial interest to the author, and it is in this field that the greatest progress has been made during the past few years. This has followed two distinct lines. In this country such studies have been restricted to neoplastic tissues derived initially from one or another of several types of aseptic tumors. Tissue cultures have been isolated from hereditary tumors of *Nicotiana* (84), crown-gall tumors of *Helianthus* rendered bacteria-free by unknown natural means (93) and crown-gall tumors of *Vinca rosea* rendered bacteria-free by heat therapy (92). All three types of tissue cultures have been shown to be transplantable and to produce new tumors in healthy hosts upon implantation (90, 92, 93). Factors involved in controlling differentiation of *Nicotiana* tumor cultures have also been studied (73, 85). The respiration of tissue cultures of tumor tissues (*Nicotiana* and *Helianthus*) has been compared with that of healthy tissues of the same plants (91). The temperature, acidity and sugar-concentration requirements of *Nicotiana* and *Helianthus* tumor tissue cultures have been found to correspond closely to those previously established (86, 87, 88) for tomato roots (38*a*).

Tissue cultures have not been successfully established from any non-tumorous material in the United States.

In France, on the other hand, even under German occupation, the study of tissue cultures from a variety of healthy (non-tümorous) materials has made great progress with many interesting results in the field of morphogenesis. These have come from two laboratories, that of Nobécourt at Grenoble (five papers) and that of Gautheret in Paris (29 papers). Nobécourt's work has dealt with the effects of nutritional factors, especially thiamin and indole-acetic acid (55, 56), on the growth, budding, root initiation and general *in vitro* development of cultures of carrot, artichoke, potato, salsify and *Scorzonera* (black salsify) (53, 54, 57). Gautheret and his students and colleagues have studied the morphogenetic aspects of the problem, especially the rôle of indole-acetic and naphthalene-acetic acids. Thus, Morel has investigated the effect of indole-acetic acid concentration on the development of tissue cultures of grape (52), has shown that such cultures can be grown indefinitely (50, 51), establishing the only true tissue culture (in the restricted sense) of a woody plant so far reported, and has used such cultures for a preliminary study of parasitism of a non-chlorophyllaceous tissue by *Plasmopara viticola* (49). Martin has studied the interrelations between colchicine and naphthalene-acetic acid as factors in the growth of cultures of artichoke (46). Camus has examined the morphogenetic effect of newly formed buds on the orientation of vascular tissues in cultures of endive (8) and has grafted buds from one such culture to another (7). Buvat has utilized tissue cultures in a study of dedifferentiation in a number of plants, especially carrot (6). Plantefol and Gautheret have studied the respiratory processes in tissues of willow and of carrot (61). Gautheret himself has published 15 papers in the past four years. These include a general manual covering the techniques of plant tissue culture (22), three papers on the effects of heteroauxins on growth of various tissues *in vitro* (26, 27, 30), two more papers dealing with tissue polarity, especially polarity in movement of heteroauxin, and the morphological responses which are believed to result from this polarized movement (19, 25), six papers on the general morphogenesis of plant tissue cultures (18, 21, 28, 29, 31, 32), a note on the extended cultivation of tissue fragments of artichoke (20), one on cultivation of very thin slices of artichoke and carrot (23) and a brief discussion of the bearing of these various findings on the growth-hormone theory of Haberlandt (24). With the exception of one paper on the development of buds on cultures of cambial tissues of elm (25), these are all qualitative. Gautheret and his students, as well as Nobécourt, lay great stress on the importance of indole- or naphthalene-acetic acid on the growth of tissue cultures, considering them impossible without either an external or an internal source of such substances. Gautheret recognizes that these substances are poisons and believes that the neophysiological irritation of such substances is necessary to prevent maturation, differentiation and loss of proliferative capacity in the cells. It is interesting to note that while tissue cultures have been maintained with equal success in the United States without external sources of heteroauxins, these have all been with tumor materials, and there is some evidence that heteroauxin may play a part in the initiation of such tumors (5*a*). If dependence on a heteroauxin is truly a differential characteristic as between tumorous and healthy tissues, it may prove to be of considerable importance. Since none of the French workers has studied any bacteria-free tumor tissue and no non-tumor tissue has yet been successfully grown by the American workers, this remains a matter for speculation. These materials present possibilities which have as yet scarcely been touched.

The problem of Plant Tissue Cultures, like all other problems, has passed of necessity through a series of stages. At the time of the author's first review in 1931 (81) one could only sketch the theoretical outlines of the field, since no really successful cultures had at that time been established. By 1936 (83) some progress had been made with roots and with cambium. In 1941 (86) the method, though by no means fully perfected, was sufficiently established that attention had begun to be turned toward applications in a variety of problems. Today the method is firmly established, methodology is no longer a matter of crucial importance, and we may hope to see the field of questions in which it may aid toward solutions ever widening. The author considers it a matter for personal gratification to have been privileged to span the decade and a half of investigation in this field and to have played a part in its development.

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THE OEDOGONIACEAE. II[1]

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INTRODUCTION

Botanical investigations, like those in other sciences, have suffered immeasurably in the last decade because of the global catastrophe that engulfed us. Relatively few papers dealing with algology have come out of the European countries at war, or from their colonies, since 1940. Reports dealing with the Oedogoniales which have seen the light of day since 1936—the majority of these are from America—have dealt largely with the taxonomy and distribution of the members of the group.

Papers dealing with the Oedogoniales as a whole include a definitive monograph of the North American species (29) and compilations (9, 13, 14). New species in the order have been described in the last decade (1, 3, 8, 11, 12, 15–17, 19–22, 24, 28, 29, 32).[2] The summary table that follows gives the present numerical status of the three genera of the order. Incompletely described or doubtful species are omitted.

Genus	Species	Varieties	Forms
Oedogonium	322	63	21
Bulbochaete	67	8	5
Oedocladium	9	0	0

DISTRIBUTION AND PERIODICITY

Certain delimited geographical areas have been explored more or less thoroughly for Oedogoniaceae, especially the genera *Oedogonium* and *Bulbochaete*. The reports on these investigations are lists of species, annotated analyses of collections, compilations of literature, detailed coverage of local species by descriptions and keys, or some combination of these. To date, since the previous report in The Botanical Review (27), such publications represent parts of China (12–14), Brazil (28), Puerto Rico (31), North Carolina (34), South Carolina (18), Florida (30), Massachusetts (6), Illinois (4), Texas and Louisiana (26a).

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[1] Supplement to article in The Botanical Review 2: 456–473. 1936.

[2] Papers that came to the attention of the writer prior to 1937 are included in the bibliography of Tiffany (29) and are not recorded here.

These reports give additional credence to the generalization that many, if not most, species of *Oedogonium* and *Bulbochaete* are widely distributed in temperate and subtropical freshwater pools, ponds, lakes and streams. The range of the terrestrial species of *Oedocladium* has been extended in the United States to include North Carolina and Florida. Couch (5) found vegetative filaments of a species of *Oedocladium* in Arkansas. Three of the nine described species of this genus are reported from India (1, 24). Only one species, *Oedocladium hazenii,* is aquatic.

Distributional data are still too meager to furnish much definite correlation of species abundance with geographical or environmental factors. There seems to be some indication of a possibility of reference to certain "subtropical", "temperate" and "ubiquitous" forms in the genus *Oedogonium.* The following paragraph (30), however, indicates the difficulty of making any definite classification on such bases.

Over one-third of the species of *Oedogonium* from Florida also occur in other subtropical areas, including Puerto Rico, Mexico, Brazil and the West Indies. Eighteen of these "subtropical forms" occur in Puerto Rico alone. On the other hand, nearly a third of the Floridan species has also been reported from each of the States of Iowa, Illinois, Michigan and Ohio; about one-fourth from Massachusetts; nearly one-half from the Scandinavian countries and Finland; and only ten species are not reported north of the thirty-sixth parallel of latitude in North America.

Most species of *Oedogonium* and *Bulbochaete* are annuals, although collections from tropical and subtropical regions indicate, as was to be expected, that times of sexual reproduction differ from those in regions to the north. In both Florida (30) and North Carolina (33) maximal sexual reproduction occurs in some species a month or two months earlier than in the North Central States. The number of perennials in the warmer latitudes may be quite large, due to vegetative persistence and to repeated formation of zoospores. Indeed, there is some evidence that an occasional species does not reproduce sexually at all.

The distribution of the species of *Oedocladium* presents an interesting problem. In continental United States the genus has so far been reported from southern States bordering the Atlantic and from West Virginia. Its presence in Arkansas (5), perhaps going back to the period before the Mississippian Embayment, suggests a relation to historical factors. The Indian species have been found at altitudes of 3,000 to 5,000 ft. in the Himalayas. In the United States *Oedocladium* grows at much lower altitudes. The terrestrial species of the genus are almost always found growing with *Vaucheria,* moss protonema, liverworts, or (in India) *Fischeriella* and *Oedogonium.*

A new terrestrial species of *Oedogonium* (*O. terrestre*) has been reported from northern India (22).

CELL STRUCTURE AND REPRODUCTION

Francini (7) described mitosis in *Oedogonium* and considered the nucleolus important in the formation of the transverse wall. Others (10) conclude, on the basis of certain chemical investigations, that some species of *Oedogonium* are more closely related to the higher plants than to other algae. They found only sitosterol in unsaponifiable material, discovered a new phytosterolin, and observed α-carotene.

Most students of the Oedogoniales have used the terms "monecious" and "dioecious" in distinguishing among certain species, depending upon whether the oogonia and antheridia develop on the same or on separate filaments. Since these words have been applied to diploid sporophytes as well as to gametophytes, Blakeslee's suggestion (2) of terms to be used in describing sexual reproduction in cryptogamic gametophytes would appear to be pertinent to the Oedogoniales. The macrandrous species of the group may then be regarded as "homothallic" or "heterothallic" (26), depending upon whether the male and female sex organs appear on the same or different filaments.

Biswas (1) suggests that a simple unbranched terrestrial form of *Oedogonium* might evolve through a poorly branched form of *Bulbochaete* with setae to a fully branched form of *Oedocladium* with setae suppressed to apical caps. *Oedocladium* would thus be considered as close to *Bulbochaete* but as having reached a higher state of evolution. Randhawa (24) contends that it is much more likely that *Oedocladium* and *Bulbochaete* arose independently from *Oedogonium*-like ancestors by acquiring a potentiality for branching.

Sen (25) reports that certain species of *Oedogonium* are important in alkaline waters in ensuring good breeding places for *Anopholes sundaicus.*

Subterranean resting "buds", responsible for perennation of *Oedocladium operculatum,* have been reported (23) in addition to the usual oospores. The intercalary akinetes are full of food, especially starch. The nodal akinetes resemble the "gemmae" first described by Stahl in 1891 for *Oedocladium protonema.* Other dormant bodies are the yellowish-brown parthenospores, described as arrested and transformed oogonia, full of starch and other foods.

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THE CHEMISTRY AND PHYSIOLOGY OF THE PECTINS. II[1]

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In the ten years that have elapsed since the previous review under the above title was written, a very considerable clarification has taken place in the field of pectin chemistry. Pectic acid has been shown (16) to consist of chains of galacturonic acid. The arabinose and galactose commonly associated with pectin preparations is, therefore, not a constituent of the pectic acid itself but rather of associated polysaccharides. Hirst and Jones (4) have shown that protracted extraction of peanut pectin with 70% alcohol removes an araban, while similar extraction of apple pectin yields a mixture of an araban and a galactan. The araban so obtained consists of chains of l-arabofuranose residues linked through the 1–5 positions. Branching occurs freely, the branches being attached through the 2-positions (4, 6). The galactan component has not been studied so industriously. Hirst, however, has studied a galactan from lupine seeds which is made up of *d*-galactopyranose residues linked through the 1–4 positions by β-glycosidic linkages.

The structure of the polygalacturonide chains has been studied by Luckett and Smith (14) who have shown that in citrus pectic acid the chains are most probably made up of pyranose anhydrogalacturonic acid residues linked in the 1–4 positions through α-glycosidic linkages.

Pectic acid possesses only a very slight reducing power and yields no tri-methyl methylgalacturonoside methyl ester after methylation and methanolysis. This indicates that the galacturonic acid chains in it are either extremely long or that the ends are in some fashion protected. The pectic acid of Morell, Baur and Link (16) yielded an apparent chain length of 8–10 galacturonic residues after methanolysis, while that of Luckett and Smith (13) yielded a chain length of 13, as indicated by physical measurements. These values for the chain length of pectic acid are greatly at variance with the estimate of 100 residues per chain arrived at by Henglein and Schneider (3) for dinitropectic acid on the basis of viscosity measurements, and are even more at variance with the chain lengths of up to 1,000 residues estimated by the same workers for nitrated pectins. It is probable that in the preparation of methylated pectic acid extensive breakdown of the chain molecules takes place. In addition, it is possible that in native pectin there may be association of chains into larger units, a theory proposed by Kertesz (11) on the basis of measurements on the rate of decrease of viscosity of pectin sols during enzymatic degredation. It is also possible that pectin may contain branched chains, similar to those found in starch (*cf.* 2) and in the gums (6), although no chemical evidence indicating that this is likely has been adduced as yet.

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[1] Supplement to article in The Botanical Review 2: 475–497. 1936.

There has long been an interest in the question whether or not a polygalacturonide, as pectin, might arise in nature by oxidation of the corresponding hexosan, in this case galactan. It would now seem unlikely that pectin arises through oxidation of galactan because the pectic acid chains possess a structure different from that of the associated galactan. In pectin the individual residues are connected through α-glycosidic linkages, while in galactan the residues are connected through β-glycosidal linkages. Similarly there has been speculation as to whether araban might arise from decarboxylation of the corresponding polygalacturonide. This is excluded in the present case, since the araban associated with pectin contains furanose residues whereas the pectin itself contains pyranose residues.

The subject of the enzymes which attack pectin has been reviewed by Kertesz (8). Kertesz refers to pectase, the enzyme which de-esterifies pectin, as pectin demethoxylase. The name pectin esterase would, however, seem to be more accurate (12). Pectinase is now termed pectin-polygalacturonase by Kertesz. Methods for measurement of pectic enzyme activity have been proposed (9, 10). Joslyn and Sedky (7) have shown that the pectic enzymes in fruit mashes can destroy 65% or more of the total fruit pectin within 24 hours.

During recent years considerable interest has been shown in the development of wider uses for pectin and pectin products. De-esterification of pectin by any of several methods has been found to result in pectic acid and pectinic acids which form gels at low sugar concentrations, whose salts may find uses (1) as aids in spray drying, for paper coatings, as thickening agents in desserts, pie fillings or puddings, as latex thickeners or creaming agents, and even as agar substitute in bacteriological media (15). De-esterification *in situ* in the fruit with the aid of the naturally occurring pectin esterase, followed by extraction of the pectinic acids, has been shown (17) to result in products of particularly high viscosity and of good film-forming properties.

While very considerable advances in the chemistry of pectic compounds have been made during the past eight years, no corresponding advance in our knowledge of their physiology has occurred. The mechanism of the formation of pectins is also a subject whose future development will be of interest.

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RECENT DEVELOPMENTS IN FUNGICIDES. II[1]
SPRAY MATERIALS—1936–1944

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INTRODUCTION

In this paper the word fungicide will have the same meaning as in the previous paper (46), of which this is a continuation. It will include only those substances which when applied to higher plants in active growth will prevent the development of fungus diseases without serious injury to the treated plants. This definition excludes disinfectants, as defined by Whetzel and McCallan (59), and all materials applied to dormant or partly dormant plant parts or to picked fruits.

The materials discussed must be beyond the laboratory stage of development. It is not the purpose of this paper to list the thousands of materials that have been tested in industrial, State and Federal laboratories. Only those will be considered that have been tested on plants in the field for the determination of phytotoxic as well as fungicidal properties. Those that have undergone only limited preliminary field tests will be mentioned but not discussed.

Bordeaux mixture remains the standard copper fungicide, but the cuprous oxides, basic sulfates, silicates, oxychlorides and others of the so-called "insoluble" group have come into commercial use, chiefly as sprays and dusts for truck crops (65). Manufacturers have greatly improved the physical properties of these materials by decreasing the size of particles.

Lime-sulfur solution and sulfur are still the standard fungicides for the control of such diseases as apple scab (*Venturia inaequalis*) and the powdery mildews (*Erysiphaceae*) affecting many species of plants. Sulfur in quantity is used for the control of peach brown-rot (*Monilinia fructicola*) and scab (*Cladosporium carpophilum*). The sulfurs now used in dusts and sprays cover and adhere better than those formerly used because they are more finely ground and because, by the use of organic "conditioners", the particles are prevented from sticking together (20). The toxicity of sulfurs for powdery mildew is so specific that it is doubtful that any new fungicide will supplant them for powdery mildew control alone. Silver compounds have shown some fungicidal value but have not come into practical use (40).

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[1] Supplement to article in The Botanical Review **2**: 586–600. 1936.

Although the coppers and sulfurs are still the important fungicides, developments of the past ten years have been chiefly in the organic field. In fact, the number and diversity of organic compounds surviving preliminary laboratory tests is such that it is difficult to determine which should be selected for field experiments. Research in organic fungicides has been stimulated by the desire of the investigators and plant growers to find better fungicides, by the manufacturers' search for new uses for their products, and by the war-shortage of copper (8, 47). The remainder of this paper will be devoted to developments in this field.

ORGANIC FUNGICIDES

In his previous paper (46) the writer stated: "At the present time none of the organic compounds or their derivatives show promise of taking the place of the standard inorganic fungicides now in use". At present none of them has come into extensive use but some of them give promise of this (8). The properties that a successful fungicide should have are set forth in detail on pages 586 and 587 in the previous paper, where it is stated that "an ideal fungicide should be toxic to the pathogen, noninjurious or even beneficial to the plant sprayed, even after repeated applications, should cause no toxic accumulations in soils, should be nontoxic to men and animals, cheap and easily obtained, nonexplosive, capable of storage without deterioration, noncorrosive, easily made and applied, conveniently handled, capable of covering and sticking well, remaining active over a considerable period of time, and be insecticidal or compatible with the insecticidal sprays without lessening its effectiveness or that of the insecticide". These requirements show why a long period of testing is necessary before a fungicide can be recommended for use on plants and especially on plant parts that are to be eaten. The questions of possible toxic accumulation in soils and possible toxicity to man are not readily answered. This is particularly true of organic fungicides because often there is no previous knowledge of their effects on man, domestic animals, plants and soils. The problem of compatibility with insecticides is complicated by the activities of entomologists in the introduction of new insecticides.

Optimum dosages have not been worked out for any of these compounds. The determination of optimum dosage takes a long time because it is dependent on so many factors, including toxicity to fungus spores, phytotoxicity, and tenacity and stability of residues, in addition to the effects of weather conditions on pathogen and suscept.

At the present stage of development organic fungicides appear to be more specific than inorganic materials in their toxicity to different species of fungi and are effective at much lower concentrations than the inorganic materials. The finished spray is not so bulky and does not form the heavy unsightly residue of Bordeaux mixture, for example. There is less tendency for direct injury to the sprayed plant but a greater tendency to interfere with normal development. For example, vegetative growth may be stimulated to the detriment of fruit or tuber formation, or the maturity and coloring of fruits may be delayed or prevented. Some of them lack the weathering properties of Bordeaux mixture, either because they do not possess inherent tenacity or because of change of composition when exposed to air and moisture. Most of them are expensive to manufacture, but this may be offset by their toxicity to fungus spores at high dilution, sometimes astonishingly high, and by large-scale production. It would seem, however, that as dilution increases there must be a point at which the quantity of toxic material on a given area of a leaf or a fruit would be too small to resist weathering. Also, with higher dilutions, even distribution becomes increasingly important, since the residues would finally become so slight that they could not be expected to disperse toxic substances outside the areas they actually occupy (23). It is possible, however, that the problem of how to deposit highly dilute fungicides in an even tenacious layer may be solved by the use of carriers in which the active ingredients are dispersed or adsorbed.

DERIVATIVES OF THE DITHIOCARBAMIC ACIDS

As a group, derivatives of the dithiocarbamic acids have shown the most promise of producing fungicides that may become commercially important. As indicated by their names, they have a high sulfur content, often 50% or more, and are sometimes referred to as organic sulfur compounds (19).

Tetramethyl thiuram disulfide appears to have been the first of the group to be tested (38); however, use of derivatives of the acid as disinfestants had previously been patented (18). This compound is highly toxic to fungi but has sometimes caused serious foliage injury when lime as a safener for lead arsenate is used with it. It has, however, controlled apple scab without serious injury (12). It has also controlled brown-rot and gray mold (*Botrytis cinerea*) of sweet cherries (43), but not cherry leaf-spot (*Coccomyces hiemalis*) (22). It has been shown to be an effective spray for the control of damping off (*Rhizoctonia* sp.) of celery (58) and tulip blight (*Botrytis tulipae*) (49). It may be considered as one of the promising fungicides of this group.

The metal dialkyl dithiocarbamates have received extensive investigation (18, 56, 57) and are at present being subjected to extensive field experiments. Of these, ferric dimethyl dithiocarbamate (Fermate) is the most promising and has shown up so well that it has been used to some extent by commercial growers. As obtained from manufacturers, it is a brown powder, only slightly soluble (120 p.p.m.) in water, which goes into suspension readily. It leaves a black residue which is undesirable on fruits approaching maturity. It burns peach foliage when used with lime, presumably because of the formation of a soluble calcium organic salt. On the other hand, it can be used with, or just previous to, oil sprays on apples without risk of injury (21, 30), an advantage not possessed by lime-sulfur and elementary sulfur sprays which cause severe injury when so used. It is unsafe to use Fermate before or just after bordeaux mixture (30), probably because of the formation of the injurious copper salt (18). It has given good control of apple scab (12, 25, 41), apple blotch (*Phyllosticta solitaria*) (10, 11, 12), apple leaf spot (*Physalospora obtusa*) (34) and apple bitter-rot (*Glomerella cingulata*) (12, 13, 30, 55) without serious injury; but, on the other hand, it has been reported as not controlling blotch and bitter-rot (34). In the control of apple cedar rust (*Gymnosporangium juniperi-virginianae*) it has surpassed the sulfur sprays now in use (25, 41, 55). It has controlled pear scab (*Venturia pirina*) without causing serious fruit russet (31) and has shown some promise as a control for pear leaf blight (*Fabraea maculata*) (49). It has been tested with considerable success for the control of brown-rot and gray mold (*Botrytis* sp.) of cherries (9, 41, 42, 43). Applications for the control of these cherry diseases and of peach brown-rot, made just before harvest, leave undesirable black residues on the picked fruit.

In the control of cranberry field rots (*Guignardia vaccinii* and *Acanthorhynchus vaccinii*) it has been superior to the commonly recommended bordeaux mixture (60).

It has controlled blight (*Coryneum beijerinckii*), rust (*Tranzschelia pruni-spinosae*) and brown-rot of peaches (63), downy mildew (*Peronospora destructor*) of onions (36), and powdery mildew (*Erysiphe cichoracearum*) of cantaloups (36); but it has also been reported as not controlling onion powdery mildew (45). It has controlled tulip blight (19), damping off (*Rhizoctonia* sp.) of young celery plants (58), carnation rust (*Uromyces caryophyllinus*) (51), black spot of roses (*Diplocarpon rosae*) (56), downy mildew or blue mold (*Peronospora tabacina*) of tobacco (2, 3, 32) and bean anthracnose (*Colletotrichum lindemuthianum*) (1). On grape it has given fair control of downy mildew (*Plasmopara viticola*) but no control of powdery mildew (*Uncinula necator*) (52).

It has failed to control early blight (*Alternaria solani*) of potatoes and brown-rot of lemons (*Phytophthora citrophthora*) (36). It has been reported as controlling anthracnose (*Colletotrichum phomoides*) (35, 61) and early blight (53) of tomato.

It has controlled cherry leaf-spot (9), but, because its residues are toxic for only relatively short periods, too many applications need to be made for practical control (22).

It appears to be compatible with the commonly used insecticides and may be applied in dust form, 10%–20% Fermate with 90%–80% filler, such as talc or diatomaceous earth (44).

Fermate has sometimes burned the skin of the face, hands and arms of spray operators, but the burns apparently have not been serious.

The lead and zinc salts of dimethyldithiocarbamic acid with solubilities of 44 and 65 p.p.m., respectively, are also toxic to fungus spores (18), but have not been tested extensively. Their residues, being white, are less unsightly than those of Fermate; but lead, because of its toxicity to man, is an objectionable residue constituent. The lead salt has controlled apple blotch (11) and the zinc salt has controlled apple scab (12, 64) and certain diseases of vegetables (27, 62) but appears to be ineffective against apple bitter-rot (64).

Disodium ethylene bisdithiocarbamate (Dithane, HE–175), another of this group, is also a promising fungicide of recent introduction (7). It is most effective when used in combination with zinc-lime (zinc sulphate and lime) (26). Used in combination with lead arsenate it has given some control of apple scab but has caused serious injury to apple foliage (12). Used alone it has given fairly good control of early blight and late blight (*Phytophthora infestans*) of potato, and when combined with zinc-lime has given remarkable control of these diseases (14, 26, 48). The combination, however, did not control apple bitter-rot (12, 13); perhaps a higher concentration than 1 pound of dithane to 100 gallons of water would have given better results. Dithane, though soluble in water, is said to form an insoluble residue on plants (7).

Morpholine thiuram disulfide is another promising member of this group which, however, has not been tested extensively (5).

DIPHENYLAMINES

Derivatives of diphenylamine have shown some promise but appear inferior to the fungicides now in use. 2,4-diaminodiphenylamine is highly toxic to fungus spores, but its high solubility makes it easily washed off by rains (17).

Thiodiphenylamine (phenothiazine) first attracted attention because of its insecticidal properties. For a time it appeared to be a promising fungicide, but it was soon found to be ineffective except under conditions of light infestation (16, 22). Phenothiazine itself is not fungicidal, but when exposed to light and water it soon becomes oxidized to phenothiazone which is fungicidal. Unfortunately it soon becomes oxidized further to compounds that are not fungicidal (16). If the oxidation of phenothiazine could be stopped with the formation of phenothiazone, or if the process could be slowed down, phenothiazine might become a promising spray for apples. It seriously injures peaches and beans, and when applied too often, delays maturity and coloring of apple fruits. Apple leaves sprayed with it become dark green and glistening. When applied on hot sunny days it burns or increases sunburn when it hits the face, hands or other exposed parts of the spray operator.

CHLORINE DERIVATIVES OF QUINONE AND NAPHTHOQUINONE

Sulfur is a constituent of the more promising fungicides hitherto discussed. Forty-two per cent of Fermate, for example, is sulfur. The most promising of the non-sulfur-containing materials is 2,3-

dichloro-1,4-naphthoquinone (U. S. Rubber 604). This material is a powerful fungicide, even at high dilutions (54), and its residues appear to remain on plants and continue active for a long time. It has given excellent control of apple scab (12) under conditions favorable to the disease, and in the control of apple bitter-rot it has surpassed Bordeaux mixture and Fermate (12, 13). It has not caused injury to peach and apple foliage and to peach fruits, but it has caused occasional spotting of apple fruits (12, 13). These spots appear directly under flecks of the spray residue and are of the same shape. Where the spray is well distributed on the fruit these spots do not appear. They are at first deep green with darker stippling and later turn brown. It is quite possible that this injury could be avoided by an even distribution of the spray over the fruit or by increased dilution. Results indicate that the dilution used, 1 pound in 100 gallons of water, might be increased without too great a loss of toxicity. The black residue is objectionable when applications are made on fruits nearing maturity, and the spray has a tendency to delay maturity and to prevent normal coloring. It has also given excellent control of both the early and late blights of potato (26, 48) and of foliage diseases of tomato (39).

Tetrachloroquinone (Spergon), though a successful disinfestant for certain seeds, has not proved consistently effective as a fungicidal spray. Its toxicity to fungus spores is probably due to the fact that it is a powerful oxidizing agent, and its failure as a fungicidal spray may be attributable to the fact that when spread over leaves and fruit in a thin layer it soon loses its oxidation property by reduction to the corresponding quinol.

Spergon has given good control of cherry brown-rot when applied shortly before harvest, but was not effective in the control of gray mold and leaf spot (22). In California (36) Spergon controlled downy mildews of onion and belladona (*Peronospora* sp.), late blight of tomato, lemon brown-rot, cantaloup powdery mildew, and damping off (*Rhizoctonia* sp.) of young celery plants (58), but in New York it did not control downy mildew of onion (45). It has not been effective in the control of apple scab and blotch or of potato early blight (53), though at one time it seemed promising as an apple spray (50).

OTHER COMPOUNDS

The copper xanthates, successful in preliminary experiments, have not survived field tests (15).

Bismuth salicylate and other salts and derivatives of salicylic acid have given excellent results in the control of tobacco downy mildew in seed beds (6), but the bismuth salt did not control downy mildew of onion (45).

Salicylanilide, recommended for mildew-proofing under the name of Shirlan, has been tested extensively as a fungicide but has not proved successful (4).

Preliminary experiments indicate the possible usefulness of derivatives of pyridine and quinoline and of phenyl-mercuric and quaternary ammonium compounds (24, 28, 29, 33, 37, 64).

With laboratory and field workers testing thousands of organic compounds annually, it seems reasonable to expect that a number of organic fungicides will be in use before another ten years have elapsed, but to what extent they may displace the coppers and sulfurs is anybody's guess. Perhaps we may have an assortment of fungicides, organic and inorganic, some generally useful and others valuable only for the control of particular diseases against which, however, they may be greatly superior to the more generally useful ones.

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THE RELATION OF WEATHER TO FUNGUS DISEASES OF PLANTS. II[1]

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INTRODUCTION

The present review is intended as a supplement to that published in this periodical in 1935 (58), and for that reason much of the previous general introduction to the subject is omitted. It is necessary to explain that the literature on this subject, considered in its widest scope, is so extensive, being approximately one tenth of all mycological and phytopathological papers, that it is desirable to restrict this supplementary review to fungus diseases, chiefly of agricultural and horticultural crops.

Any serious paper on crop diseases almost always has some reference to weather relations or to one or more aspects of this subject, while purely mycological papers frequently include some study of the relation of weather factors to the life cycle of the organism involved. Nevertheless, much of this material is incidental to the main research, and as a result such literature finds small space in the present review. A comprehensive picture of the research on this subject carried out in the last decade is gained from the literature which exclusively deals with one or other aspects of the subject. From the point of view of general training, it is satisfactory to note that many textbooks now deal with this subject in some detail (248). It will be appreciated that in order to understand the complex relation of weather as a whole with the epidemiology of economic crop diseases, the separate component parts of the weather must be analysed in some order. In the following pages will be set out the effects of the weather factors on the production, survival, spread and germination of spores and other structures of fungi and on the initiation and progress of the epidemics they cause.

PREDISPOSING FACTORS

Production of the Means of Dispersal

Most of the information on the effects of environmental conditions on the production of various fungus spores concerns those forms causing aerial diseases. Certainly some of the earlier papers touch on soil organisms from this aspect, but many of the statements are conjectural and based on the similarity of their life histories to those of the few organisms so far investigated. This shows that there is need for research on the biology of many soil organisms.

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[1] Supplement to article in The Botanical Review 1: 497–516. 1935.

Temperature. Temperature is known to affect markedly the rate of infection of host plants and the progress of disease. The incubation period of rust diseases, that is, between initial infection and production of fructifications of the rust, has frequently been reported as affected by differences of temperature. For example, the uredospores of *Puccinia glumarum* are formed at 15° to 17° C. with most races, a temperature not much above that optimum for uredospore germination (230). In Russia a maximum day temperature of 24°–25° C. and a minimum night temperature of 2° C. promotes abundant sori production. The sori ripen quickest at 20° C. (172). In *Puccinia graminis tritici* low temperatures, such as 10° and 0° C., increase the incubation period, favour abundant uredosori formation in some forms and stimulate teleutospore development. These features may be associated with the fact that the mycelium of this rust present in infected plants is as resistant to low temperatures as the host itself (152). In Germany it is claimed that the ripening of perithecia of *Venturia inaequalis* is accelerated by the warmer spring temperatures after March 1st, particularly when the mean daily temperature aggregate reaches 105° C. by the time of perithecial maturity. Records of the pre-ripening period showed a constancy over seven years (104).

Humidity. As might be expected, humidity affects the production of fungus spores, whether in perithecia or in open fructifications. It does not follow that high humidities are always favourable. *Peronospora destructor* on onions (260) and *Bremia lactucae* (181) require 100% relative humidity for development of the conidiophores and conidia, the minima being, respectively, 90% and 96%, while it is reported that conidial formation of *Sphaerotheca humuli* v. *fuliginea* in Japan is reduced at 100% but abundant at 93%–96% relative humidity (91). This reversal of the usual relationship may be explained by the theory that this ascomycete is very sensitive to oxygen requirements, for with a saturated atmosphere oxygen will be reduced. The humidity balance is sensitive especially with reference to spore germination. Sporangial development of *Phytophthora infestans* is checked by quite brief fluctuations of 5% or more of the relative humidity, the detrimental effects being worse than those caused by much wider fluctuations in temperature (183). In many cases actual water is required for production and emission of the ascospores of very many ascomycetes and this is provided by rainfall. For example, discharge of ascospores of *Nectria galligena* in apple canker is closely associated with the volume of rain and not with other meteorological factors. Leaf scars and other wounds are penetrated in these wet spells, and control may be effected partly by pruning in cold dry spells (19, 167). However, in some cases this production of spores is favoured more by alternating periods of wet and dry conditions than by continuously wet periods, and has been confirmed for *Erysiphe graminis* (29) and *Gnomonia ulmea* (181). Presumably, the final stages in spore production and abscission require a temporary dry condition, and the second supply of water will serve for production of a second batch of spores after the first have been dispersed. The presence of rainwater is also necessary for active budding-off of conidia of *Venturia pirina:* after a good rainfall the surface of infected twigs, which have carried dormant mycelium over the winter, is "literally bathed" in conidia, some of which are drawn in between the scales of swelling buds and serve as a future source of scab (28).

The time of production of spores is another aspect which deserves mention. It is obviously necessary for the optimum conditions for spore production to occur at the right moment and also necessary for them to remain so for as long as possible. In confirmation of this, conidiophores of *Podosphaera leucotricha* are produced in five days (14), but sporangiophores and sporangia of *Peronospora destructor* (260) and *Pseudoperonospora humuli* (257) are formed and matured in one night or in 24 hours. Yarwood has established the fact that the asci of *Taphrina deformans* are formed in the evening of one day, divisions take place overnight and the ascospores mature and are discharged at 8 P.M. on the second day (259). The conidia of other ascomycetes, *Erysiphe polygoni* and *E. cichoracearum, Sphaerotheca pannosa* and *Podosphaera leucotricha,* however, are formed and abstricted during the day (33). Thus failure of the optimum conditions to coincide with the particular stage of development which they normally favour leads to cessation of growth of the fungus.

The manner in which humidity assists in the primary dispersal of sporangia of certain downy mildews is most interesting. In periods of desiccation, presumably of short duration, the sporangiophores of *Peronospora tabacina, P. parasitica, P. geranii* and *Plasmopara halstedi* become distorted, but on the return of higher humidity they straighten out with sufficient force to dislodge the sporangia to some distance; the latter then float away in air currents (186).

Survival of Fungi

The survival of fungi is affected by many factors, such as temperature, moisture content of soil and air, spore structure, light, host stimulation and cultural processes. Many of these factors have been analysed in considerable detail in the earlier papers, and it is only the recent literature that requires summarising.

Moisture. There is little doubt that moisture has a major effect on survival, since it is often found that while the spores, *etc.*, may survive quite low temperatures if they are relatively dry, they soon succumb if wet. There are numerous instances of this fact. Uredospores of *Puccinia dispersa* will survive – 26° C. dry, but rapidly die when moist (133); the thinner-walled conidia of *Bremia lactucae* (209) germinate reasonably well after 16 hours at only 16% relative humidity and the thin-walled conidia of *Piricularia oryzae* resist cold better when dry than when wet, germinating abundantly at low temperatures after being frozen at – 10° C. (1). The conidial stage of *Glomerella mume* in Japan survives – 10° C. for 80 days when moist but 260 days when dry (232). The two latter tropical disease fungi must be conditioned to both dry and hot conditions, and therefore it is not surprising that they survive adverse temperatures when dry. Survival potentials of smuts as measured by spore germination after storage in herbaria has shown that the Tilletiaceae survive longer than the Ustilaginaceae. The bunt species, *Tilletia caries* and *T. foetida,* survived 18 and 25 years, respectively, *Ustilago avenae* and *U. hordei* 12 and 13 years, *Entyloma dahliae* 10 years. It was presumed that maturity at the date of collection was correlated, to some degree, with these results (54), and there may also be morphological or other differences between the two orders to account for this.

Apparently, the sclerotia of basidiomycetes, such as *Corticium,* are more resistant to adverse environmental conditions than those of ascomycetes, such as *Sclerotinia,* and immersion in water causes a more rapid loss of viability than when left dry (178). No explanation of this fact is forthcoming, and it is open to research to discover whether this is due to structural and physiological differences or to the existence of ecotypes. The sclerotia of *Sclerotinia sclerotiorum* are long-lived enough, lasting 11 years in dry soil (21). In comparison with this, in the U.S.A. the sclerotia of the moniliaceous fungus *Phymatotrichum omnivorum* die rapidly in air-dry soil and are not favoured by very low or high soil moisture content. At a moderate soil moisture content, such as 20–40%, they survive five years (234). With really thin-walled conidia, such as those of *Phytophthora infestans,* it is not surprising that these lose their viability in 30 minutes if allowed to dry, while they stand even high temperatures of 35° C. for four hours, and low temperatures of 1°–2° C. for 48 hours, if kept moist (180). Exposure even to 76% relative humidity destroys the conidia in one hour, and exposure to 86%–94% humidity impairs the germinative capacity (183). It is surprising, therefore, to find that sporangia of *Pseudoperonospora humili* survive a severe drought for one month in the field (146), for there is little evident structural difference between the conidia of the previous fungus and this one. These sporangia (or conidia, according to the method of germination) also lasted nine days on drying, cut hop leaves at temperatures of 18° to 35° C., though death followed in 16 days. On the host tissues, at low temperatures of 5°–10° C. in the dark, the sporangia last 40 days, but once they are separated from the hop leaves they die rapidly. The effect of the host tissues is also shown in increased activity of zoospores in water suspensions (146). It is understandable that oospores of the Peronosporaceae should require ample moisture for germination and that they should be able to survive unfavourable conditions for long periods, owing to their very thick walls. There still remains much to be learned about the dormancy, germination and general biology of these spores, however. Two recent researches on this particular subject concern *Peronospora destructor* and *Pseudoperonospora humili.* The oospores of the former were found to germinate progressively better the greater their age after four years (144). The oospores of the latter germinate over a wide range of temperature after wetting for four to 16 days and require a dormant period of several months. They also survive several years of dry storage at about 15° C. or at – 18° C. (146). In addition, it was observed that oospores of *Sclerospora graminicola* could survive eight years when dry (233).

Temperature. The influence of temperature is nearly as important as that of moisture, and acts in various ways. It is a generally accepted principle that dormancy is initially broken by the increase of temperatures in spring. In some instances, certain spores cannot survive in a dormant condition, but either germinate within a short period or die, *e.g.*, the conidium of *Erysiphe graminis* (29). The perithecia of this fungus are claimed to carry it over the summer rather than over the winter (29), but this assumption is at variance with the opinions of other workers (60) who consider the perithecia as the over-wintering phase. Although the conidia of this fungus are reported as germinating at temperatures up to 35° C. and even in a dry atmosphere, it seems likely that summer conditions in Canada are liable to kill the conidia. On the other hand, in more temperate climates survival of this fungus over the summer is likely to be independent of the perithecia; hence, it is suggested that in hotter climates the perithecia serve to carry the fungus throughout the year, in temperate climates only through the winter. In another hot country, India, it has been found that the conidia of apple scab (*Venturia inaequalis*) lose their germinative capacity very quickly, owing to the high temperatures, and only freshly formed spores generated in spells of lower temperatures of 10°–12° C. are able to germinate (175). No doubt, the perfect stage serves to perpetuate this fungus over the hot summers in India.

It is now well established that the uredospores of cereal rusts have different temperature requirements than those of the teleutospores, and this fact may be correlated with the annual epidemics, the source of the inoculum being in some cases local and in other cases many hundreds of miles distant. Black rust of cereals survives hot climates quite readily by means of its uredospores, but there is a limit to this, since while 8% uredospores survive 44° C. for two days, only 1% survive 50° C. and none at 60° C. (109). The summer temperatures may reach 40° C. (104° F.) in Minnesota and even 47° C. (117° F.) in the shade in India; hence, any prolonged period of very hot weather in those countries is liable to kill the majority of these spores.

In North Carolina the winter temperatures are such that by February no viable uredospores of *Puccinia graminis tritici* remain, in spite of the fresh infections which take place from October to December. The sources of infection each year, no barberry being available as the alternate host, are drifts of wind-blown uredospores from south-westerly States in which these spores can over-winter (235). In north Italy uredospores of this same rust occasionally survive on self-sown wheat or grasses, but usually the winter temperatures are too low to permit this. Therefore, the rust is carried over as teleutospores which infect the common barberry which is common in the alpine districts of Italy (214, 215). This is also the case for black rust in Austria, where also *P. triticina* over-winters in the form of mycelium (6), but *P. dispersa* in the form of uredospores (245).

Many spores which are resistant to low temperatures, as for example the conidia of *Sclerotinia laxa* and *S. fructigena* which survived six months on fruits stored at – 14° to – 18° C., fail to survive for long if they are exposed to alternating cold and warm weather in the open air during winter (25). If conidia of *Sclerotinia fructicola, Plasmopara viticola* and *Venturia inaequalis* are frozen dry, by first lining Dixie cups with ice and then adding dry spores, they will survive – 10° C. very readily and even – 40° C. The galls of *Gymnosporangium juniperi-virginianae* can also be frozen to such temperatures, and remain quite viable over a year, but if a water suspension of the separated spores is frozen, then viability declines in three weeks (84). Some spores can be accustomed to low temperatures by a hardening process, and data on this subject are doubtless of value in countries where very severe winters occur. If the uredospores of *Puccinia graminis* are hardened first for ten or more days at 0° C., they survive very low temperatures such as – 29° to – 40° C. much better than unhardened spores. But such hardened spores were more liable to be killed by daily fluctuating temperatures above and below 0° C. than unhardened spores (152). The uredospores of crown rust of oats (*Puccinia coronata avenae*) are favoured by low temperatures (5°–10° C.), but as the temperature rises fewer spores survive, until above 15° C. at any humidity none survives. These spores are not found to perpetuate this rust from year to year in the U.S.A. (197). Longevity of uredospores, other survival factors presumably being optimum, is influenced by the date and temperature of formation. Those of *Puccinia triticina* survive longest when formed at 15°–20° C. during the month of May (206).

Cladosporium fulvum has long been known to be favoured by high temperatures and high humidity, but while the conidia are killed by a temperature of 46.5° C. (115–116° F.), they apparently can withstand severe winters and remain viable for a year under really severe adverse conditions (81, 82, 113). Other studies confirm this resistance to freezing of *Cladosporium, Botrytis, Penicillium* and *Fusarium.* In countries where the tomato is grown commercially in the open this resistance is an important factor. Many fungus hyphae are no doubt no more resistant to adverse conditions than thin-walled sporangia and conidia, but such hyphae as those of *Rhizoctonia* and *Ophiobolus* are markedly so. The hypha of *Ophiobolus graminis* was found to survive both 34° F. of frost and marked alterations of temperature between – 20° and 70° F., both in culture tests and in the field (54, 60). However, the mycelium and sclerotia of *Phymatotrichum omnivorum* do not survive – 13° C., even for 24 hours, but while growth is inhibited the fungus is not killed at 5° C. It is considered, therefore, that this disease will be limited in the U.S.A. to areas south of a line marking the minimum air temperature of 23° F. (50). Garrett, however, considers the survival of sclerotia of root fungi to be a complex of many factors, of which the microflora as a whole, soil texture, as well as moisture, temperature and acidity, have their part to play (69). The same is true for spores and mycelium, and Garrett sums up thus: "the greater the physiological heterogeneity in a population of resting spores, the longer is the probable survival period of the parasite" (69). The chief cereal root fungi, *Helminthosporium sativum, Fusarium culmorum* and *F. graminearum,* amongst others, were found in Canada (Alberta) to over-winter naturally as mycelia and conidia. Young germ tubes re-grew after being frozen solid overnight at 6° F., while cultures survived 17 days between 0° and – 50° F. or continuous freezing for three months or repeated alternate freezings and thawings over two months. *Helminthosporium* survived better on the soil surface, but the fusaria better when buried (60).

Gibberella saubinetii is known to grow at a wide range of temperatures, and attacks wheat at high and maize at low temperatures, following a correlation of cell susceptibility associated with insufficient carbohydrates at those respective temperature ranges. It is therefore surprising to find that this fungus when present in seed barley dies sooner when the grain is stored in the laboratory, while other fungi infecting that grain die more readily when stored in a cooler seed house (212). No evidence is available to explain this. It has already been mentioned that conidia of *Phytophthora infestans* survive high and low temperatures when moist (180), but it has now been shown that water suspensions of zoospores and sporangia of this fungus can be frozen to –6° C. for 24 hours or subjected to fluctuating temperatures (min. of –12° C.) for 14 days, and yet those spores remain viable and able to infect potato leaves (23). In most countries where epidemics of blight occur, it is most unlikely that such low temperatures occur often in spring and at all in summer, but it suffices to indicate that those falls in temperature that are likely to occur will not kill these spores, as other work had already indicated (180). It is possible that the loss of viability of blight conidia, according to Murphy (168) and others, was due to a high loss of water from the conidia or zoospores accelerated by high temperatures. Blight has been reported as over-wintering in fields in which a tomato crop had been grown, and it was presumed that the survival was made possible by mycelium present in tomato debris (17). Another instance of probable adaptation to low temperatures is afforded by the uredospores of *Puccinia glumarum* which if produced in the winter months have a higher infective power and remain viable longer than if produced in the summer. Incidentally, it should be noted that Straib considers that over-wintering of this rust is less dependent on the winter hardiness of an infected variety than on the length of life of the old, pendant, autumn leaves as it is affected by soil, weather and nutritional conditions (228). It is also claimed in Italy (44) that the early produced uredospores of cereal rusts do not infect summer-sown wheat but do infect the autumn-sown crops and thus must have a dormant period. This has not been supported elsewhere.

Concerning the survival of smut spores on or in soil, there is a differential response according to the separation of spores. For example, only 11% of *Tilletia caries* spores survive as separated spores in surface soil, but 29% of spores survive if in balls and

45% survive if in whole heads. Further, survival was better on the surface than when the spores were buried in the soil (86). Other smuts behave similarly: *Ustilago zeae* spores survive best on the soil surface, reasonably well if buried in dry soil but poorly in wet soil (16). More recent tests gave these results: spore survival in pure sand at 10 cm. depth was 100% in three years and 50% in five years; at 20 cm. depth it was 10–25% in eight years (128). There was no evidence here whether depth or age was the major factor in reducing the survival rate. It is claimed the viability decreases progressively by using loam, clay and humus instead of sand. *Urocystis tritici* survives best buried deeply, but even there the spores gradually die (80).

Sclerotia of *Sclerotium rolfsii* also vary in their viability when buried in soil: on the surface, at four inches depth in moist soil or at two inches depth in very wet soil survival was satisfactory, but at five to six inches depth in wet soil mortality was very heavy (51). It would seem from the above examples that moisture, particularly if in excess with a consequent lack of aeration, causes the fall in survival rates of many soil inhabitants or invaders.

An interesting analysis of geographical distribution of various parasitic fungi showed that this may be closely correlated with the temperature ranges of the fungi as determined under laboratory conditions (225). For example, *Diplodia zeae,* like many other maize fungi, has a relatively narrow temperature and geographical range, no growth occurring at 10° or 35° C. The species of fungi growing at high temperatures (35° C.) are widely distributed in the tropics, while those with good growth at 10° C. are found in the north temperate regions. *Botryosphaeria ribis* has one of the widest temperature and geographical ranges (225).

Light. Although it may be a little out of place here, it seems desirable to discuss the effect of light, since in addition to its influence on the germination of spores and on infection, it also affects the survival of fungi. A very thorough review of this subject up to 1935 has been published (217), and the following résumé from that paper covers the research up to that date. Both visible and ultra-violet light might prove lethal to mycelium or to spore structures or might stimulate either to growth. The effect depended on the intensities and the wave-lengths involved; also the species of fungi varied individually in their reactions. Small doses of irradiation did not stop growth processes but might have stopped cell divisions. Sporulation and even the shape of spores could be influenced in some instances by irradiation. In general, long exposures to ultra-violet light decreased spore production, but short exposures stimulated the process. The germination of spores was independent of moderate intensities of light, but strong irradiation inhibited it. Pigment in cell walls tended to protect the spores. The lethal effect of irradiation was decreased or slowed down at low temperatures. X-rays and radio-active rays were considered to be more harmful than long waves.

Since 1935 observations and experiments have again confirmed the harmful effects of strong sunlight on sporangia of *Pseudoperonospora humuli,* death following exposure for 24 hours (146) on conidia of *Cladosporium fulvum* (81, 82, 113) and on teleutospores of *Cronartium ribicola,* which were still viable, however, after eight hours exposure (99). Uredospores of *Puccinia glumarum* were found to germinate more rapidly in subdued daylight than in darkness above 15° C., but there was no difference below 15° C., while infection could take place in darkness (230).

Low pressure mercury vapour lamps have been in use for some years to control the development of moulds on stored foods in bakeries, refrigerators, transport waggons, *etc.,* the ultra-violet light emitted being strong enough apparently to stop all growth (73, 77, 96, 134). The peak of lethal action is about 2,250 Å, an amount which it is stated is not found in sunlight at the earth's surface (96). To kill spores and sporidia of *Ustilago zeae* wave-lengths between 3,022 Å and 3,130 Å are required (134). Ultra-violet light was found to inhibit growth of *Sclerotium rolfsii* and *Macrosporium solani,* but after ten hours exposure the fungi recovered and grew normally. An explanation is advanced in the theory that growth hormones become concentrated in the bulbous mycelial tips, so that after a time their presence overcomes the inhibitive effect of irradiation (263). The uredospores of *Puccinia graminis tritici* are only slightly protected by a small amount of pigment, less than the teleutospores possess, and this may explain their liability to be killed by strong sunlight; 10% were found to survive 270 hours of exposure to 500–1,500 foot candles and 75 hours at 7,000 foot candles, but none survived 270 hours at 7,000 foot candles. The sunlight in midsummer in Minnesota is stated to reach 10,000 foot candles; hence, the expectation of survival in such weather is negligible (109). Similar results have been obtained with uredospores of *Phragmidium mucronatum* (37). Viability of some spores declines more rapidly with white than with red or blue light (109).

Smith's view that some of the effects of unfiltered ultra-violet light were really due to heat has been supported by other workers (37, 68). Research on the effects of ultra-short waves is yet without conclusive results, many organisms being unharmed. While some organisms were killed by exposures to short (8–40 m.) and to ultra-short waves (5.2–10 m.), this effect was removed when the temperature was reduced by using a water-jacket. Apart from that factor, the lethal effect was more rapid the greater the density of the organism and was greatest inside infected cereal grains; hence, it was suggested that such a method might be developed for disinfecting cereal seed grain (239).

It is reported that strong sunlight in Japan depresses the size of the lesions on rice caused by *Helminthosporium oryzae* and by *Piricularia oryzae* (111, 112, 169), but, in view of other records of the effect of temperature, and in the absence of contrary evidence, it seems more probable that the direct cause of this fact is the high temperature associated with the strong sunlight. Use has been made, especially in the Punjab, of the high temperatures associated with the sunlight in May and June in order to control *Ustilago tritici* on wheat. Infected grain is soaked in water for four hours and then exposed to full sunlight for another four hours. Smut was reduced from 15% to nil and from 18% to 0.3%. The maximum shade temperatures in the Punjab approximate 120° F. (140).

As stated by Smith (217), the size of spores is influenced by light, for Harter reports that conidia of *Fusarium caeruleum* and others, on standardised media, are longest with the maximum amount of light and shortest in the dark, a medium length occurring with three hours of daylight (89). This is also confirmed for uredospores of *Puccinia graminis tritici* f. 15, which were longest with 301 foot candles, shortest in glasshouse conditions and intermediate with artificial light sources (152). This is, of course, the reverse of the effect on *Fusarium caeruleum*.

While work in Italy has shown that ultra-violet light inhibits the formation of pustules of *Uromyces appendiculatus* (211), low light intensity delays formation of uredosori of *Puccinia graminis* (152);

the latter conforms with the theory that with poor light conditions the nutrition of the plant is unbalanced, and not enough carbohydrate is manufactured to feed both host and parasite. This was pointed out by Brown (22) who gives a number of references to work on this subject and who indicated that the general effect of light upon a host is to increase its susceptibility to leaf parasites, there being an optimum period of illumination. He quotes Forward as finding that *Puccinia graminis tritici* uredosori are fewer and slower in development when the host is in the dark and that with poor light there is a tendency towards hypersensitiveness in hosts that are normally congenial to the parasites. This has been supported by others for this and other diseases (29, 85, 90, 247). The effect of light on the infection of various hosts by seven rust fungi was tested, and infection by five fungi was found to be reduced by darkness, but two (*Puccinia triticina* and *P. antirrhini*) were unaffected (87). In *P. triticina* it was reported that some wheat varieties have their resistance increased by curtailment of light, others the reverse (92). Later, Hassebrauk supplemented this by stating that in the early stages of infection the resistance of moderately, and sometimes of highly, resistant varieties was reduced by poor light, low temperatures and high humidities, whereas in the middle phase only the appearance of pustules was delayed, and in the latest phase resistance was actually increased (93). Low temperatures will also reduce the resistance of barley to *Helminthosporium gramineum* (114). Again, while infection by one strain of *Puccinia lolii* was reduced by decreased light intensities, another strain was not (87).

Varieties of wheat vary in the stage at which they may be susceptible to various diseases, and often one variety is susceptible in the seedling stage and not in the mature stage. It is thus interesting to note that certain wheat varieties, which beyond the seedling stage appeared resistant to *P. graminis tritici* with normal sunlight, become as susceptible as the seedling stage when shaded (88). Since high temperatures of 29°–33° C. conferred the same degree of resistance as intense sunlight, this situation may involve intense sunlight raising the temperature of the host tissues to that which confers resistance, rather than a lethal action independent of heat. That photoperiodic conditions should affect the degree of infection of plants is to be expected, even though the action is an indirect one involving the metabolic balance between host and parasite. Variations in the degrees of infection with differences in the length of daylight suggested an experiment with *Cronartium ribicola* in which inoculated plants were exposed to daylight for periods from less than nine hours to more than 17 hours (165). The results were:

No. hours exposed:	above 17,	17,	16,	15,	14,	13,	12,	11,	10,	under 9
Percentage leaf infection:	0,	5,	10,	50,	75,	100,	40,	10,	5,	0

From this Moshkov suggests that immunity from fungus parasites may depend upon changes in the leaves induced by photoperiodic conditions. This seems likely up to a certain point and would agree with the theories of Brown and others. But while an increase up to 100% infection at 13 hours exposure to daylight may be explained on this basis, the progressive decrease in percentage infection above 13 hours is hard to explain except on a basis that the longer the daylight the greater the accumulation of heat or lethal effects of sunlight, neither of which seems reasonable.

Spore Dispersal

In discussing this subject, which is a most vital factor in the epidemiology of any disease, it is necessary to consider it from many angles. Wind and wind-blown rain dispersal of the spores of plant pathogens will normally occur only in the spring and summer months, since, as already discussed, the spores or mycelia which survive the winter period initiate the primary summer, conidial phase. It is true that a number of fungi, chiefly of importance as responsible for human mycoses or allergic troubles, may be active over a great portion of the year, indeed, over the whole year as in the case of *Aspergillus,* and for these wind dispersal is important. It is interesting to note that the genera which have species of interest to plant pathologists were found to have spore production and wind distribution mainly in the spring and summer months, whereas other allergic fungi tend to be distributed over a longer period (13, 38, 48, 49, 188). Though rather foreign to this review, it is of interest to note that the abundance of *Alternaria* and *Hormodendron* spores in analysis of air was correlated with the abundance of *Ambrosia artemisifolia* (48).

The earlier contributions to the study of wind dispersal of spores tended to magnify the distances to which spores were distributed, but recently the view has been expressed that many diseases normally have only local distribution. A comprehensive review of this subject by Craigie (40) may be utilised as a background for discussing the various factors involved. The earlier work, partly covered by other reviews (57, 58), is surveyed by Craigie. Emphasis has been made on the necessity for a quantitative survey of the spore content of air (40, 78, 79, 222), but such work should be related also to the original spore concentration on the host. For example, Craigie cites the prolific spore production of a barberry bush which may release at any one discharge, under optimum conditions, 70,000,000,000 aecidiospores of *Puccinia graminis.* A fructification of *Polyporus squamosus* may yield 100 billion basidiospores. There have been numerous calculations of this nature of the productivity of fungi (136), but, while there have been detailed mathematical treatments of the dispersal pattern (78, 79, 222), no attempt has been made to correlate the density of a spore inoculum with the observed patterns of spore dispersal at regular distances from that inoculum and with certain environmental states, specifically with certain wind velocities. Stepanoff (222) has certainly drawn attention to the need to take into consideration the abundance of spores at the source when evaluating the danger of this source of infection, but he did not attempt the suggested correlation, which might eliminate the need.annually to trap spores at regular distances.

Height of dispersal. Up to 1935 the highest record of vertical spore dispersal was approximately 20,000 feet, but since then the U.S.A. Stratosphere Exploration (1935) results demonstrated that fungus spores and bacteria were present in the air between 36,000 and 70,000 feet (223, 224). Meier and Rogers tested these organisms for viability, and recovered *Macrosporium tenuis* as well as species of *Penicillium, Aspergillus* and *Rhizopus.* These are organisms the conidia of which are small and might well be carried further aloft than many rusts. Also the ubiquity of these allergic fungi (13, 38, 48, 188) may explain their predominance over other fungi in high altitude analyses. Little information had been published prior to 1936 on the conditions at very high altitudes, but Steven's results (224) show that the temperature at about 65,000 feet was – 78° F. Quartz tubes containing seven mould fungi were exposed at the maximum height reached (72,395 ft.), and in spite of the greatly increased danger of death from ultra-violet rays and from low temperatures (*i.e.*, freezing at least of – 110° F.), five, including *Helminthosporium sativum,* grew normally on return to the laboratory. Unfortunately, no details were given as to the humidity conditions in the exposed quartz tubes: if thoroughly dry, the spores might well survive longer than if moist. Meier and Emmett, following these results, subjected the five fungi which survived the stratosphere exposure to a trial in test tube cultures in which they were exposed to – 78° C. (– 108° F.) for 200 hours: all survived and grew, but again it was not stated whether they survived as spores, mycelia or both (224).

Various rusts were collected in the Nanga Parbat region at heights between 7,500 and 13,500 feet (240). Presumably, conditions would approximate those of the general Himalayan range where even in summer months 53 degrees of frost are common. It is not surprising to find, after this, records of spores of fungi, such as *Peronospora destructor,* surviving at 1,500 feet (176). Since conditions more drastic than the foregoing are unlikely to occur in reasonable proximity to normal areas of vegetation, MacLachan's results with *Gymnosporangium juniperi-virginianae* are readily understood (145). He found that basidiospores of this rust could spread as far as seven to eight miles and were viable up to 2,000 feet, but while the spores were killed within one day in completely dry air or with temperatures over 30° C. at any humidity, the conditions prevailing during wind dispersal were favourable to their survival. The falling-off of spore concentration the further they become dispersed was fulfilled for this rust, and eradication of red cedars within a radius of one to two miles was considered ample to protect pomaceous hosts from infection. Other papers also deal with height of spore dispersal (40, 185, 218). The reduction in spore numbers increases with increasing altitude, as previously reported, but within the first 5,000 feet it is considered that there is as much chance of obtaining a large number of spores at one altitude as at another (40).

Distance of dispersal. While it is true that a number of fungi are distributed by winds over vast distances, the danger of such dispersal invalidating quarantine regulations is considered to be small (142), since only 10% to 25% of parasites covered by those regulations have more than local air dispersal. There are exceptions, no doubt, and unusual winds are known to cause exceptional dispersal. Allergic moulds have been carried several hundred miles in the eastern U.S.A. at concentrations a hundred times the usual (47, 48), while pear scab dispersal was found in Germany to be influenced markedly by the course of a cyclone, the deep depression in its van creating the necessary conditions for ejection of ascospores and their dispersal in the side currents created by the cyclone (95). In America, Canada and India the cereal rusts' dispersal involves vast distances, in spite of the relatively large size of the spores. For example, Craigie gives numerous instances of the dispersal of cereal rusts over 200, 400 and even 800 miles, often despite considerable natural barriers, such as mountain ranges, lakes and forests (40, 41). That a fair proportion of the spores in the spore showers brought by strong winds from the United States are viable is certain from the fact that crops become infected in direct association with these showers. Crops in Manitoba are infected first, then those of East Saskatchewan and lastly those of Alberta. The work of Mehta and others in India has also given ample evidence of the great wind distribution of black rust spores (148–150). *Cronartium ribicola* is recorded as spreading southwards in California 100 to 125 miles, but sometimes even up to 400 miles. The distribution was found to take place mainly in the upper air strata, and charts of the upper air movements facilitated reasonably accurate forecasts of the southward spread of this rust to *Ribes* in California, outbreaks on this host occurring when the humidity favoured infection (160, 161). What seems likely is that certain geographical formations favour long distance winds of considerable velocities with which this wide dispersal of rusts is associated. Evidence has also been presented of the dispersal of fungus spores 400 miles out to sea from California, the numbers collected falling off with increase of distance from the coast (115, 194). Other studies also have been made (205, 221).

There are many instances of short distance spore dispersal, too numerous to quote fully. Downy mildew of tobacco is distributed by wind, and the conidia of *Peronospora tabacina* have been blown over Lake Erie, a distance of 30 miles. In Holland two experiments demonstrated that loose smut of wheat could be spread by strong winds which carried the spores at the height of the flowering heads when the flowers were open. Light winds allow the spores to be carried in vertical air currents and so lead possibly to a greater distance of spread (182). In take-all of wheat the ascospores are ejected only during or just after rain, and dissemination by wind is likely to be only local, a matter of a few hundred yards, unless the crop is very young (202, 203). The ascospores of *Sclerotinia sclerotiorum* may be blown several miles, however (21). Splashing rain has always been recognised as an important method of spore dissemination, and recent work has verified this factor in the epidemiology of apple blotch (*Phyllosticta solitaria*) (195) and tulip fire (*Botrytis tulipae*) (9), to take only two cases from many others. While on this subject, the possibility of wind-blown infected leaves serving as a source of infection should not be overlooked, as pointed out for apple blotch (195).

Mechanics and mathematics of dispersal. The problem of the rate of fall of spores is, on the whole, a purely theoretical one, since in nature there can be few occasions when air movement is so restricted as to permit application of Stokes' Law to the measurement of the fall. Gregory (79) has reviewed the subject very thoroughly, has brought together the tables of spore dispersal published by many investigators and has subjected the whole to mathematical analysis. He considers that attempts to calculate dispersal can be done only with reference to non-turbulent air movement and are inapplicable except at the earth's surface. One such theoretical aspect may be mentioned, namely, Buller's claim that elongated spores assumed a horizontal position when falling, which affected the rate of fall. This has been shown not to be necessarily true, since the ellipsoidal conidia of *Erysiphe graminis* and *E. polygoni* fell equally in the horizontal and vertical positions (261).

Any mathematical study of spore dispersal must take into consideration the mode of detachment of spores. Stepanoff (222) divided fungi into three groups: (*a*) those with spores readily detached by winds and light air currents, (*b*) those separated and carried through the air with difficulty and (*c*) those not disseminated by air. Most fungi can be placed in groups (*a*) and (*b*), and to these Stepanoff gave the title "anemochores". Dobbs (46) has classified fungi for their mode of dispersal, and here we are concerned only with his "passive" dispersal group. This was divided into (*a*) spore retainers, (*b*) spore drop-shedders, (*c*) dry spore shedders and (*d*) spore shedders (most fungi). Again, the only groups which concern this discussion are the "dry spore" and the "spore shedders". Stepanoff investigated the rates of fall of various spores and found that this depended *inter-alia* on the size and structure of the species studied.

Stepanoff also constructed a formula to determine the lines round a source-focus at which equal numbers of spores are deposited. Gregory (79) considered it more appropriate for dispersal calculations to suppose that spore clouds were held in suspension in the air but were diluted by air eddies in the course of the major movements of the air. He gives many figures showing the falling-off of spore concentration with increasing height and with increasing distance from the source of inoculum. For the decrease in spore load at increasing height he uses formulae for eddy diffusivity used in meteorology. The reader is referred to his paper for full details, which cannot adequately be covered in the present general paper, and in which a full bibliography of 89 titles is given, though the more outstanding papers of immediate interest to pathologists are listed here (35, 61, 125, 143, 184, 220, 265).

Another aspect of aerial infection is that of physical barriers. As an example, it has been shown in the case of blind seed disease of ryegrass (*Phialea temulenta*) that even though conditions may be optimum for production and release of ascospores, infection of the flowering heads may be impeded by a densely laid crop. When the soil is thinly covered by young ryegrass, adequate air dispersal of the ejected ascospores from the apothecia on the soil surface becomes possible (179, 253). Further, it is not readily appreciated that even soil fungi may be wind-distributed, and Garrett (63, 69, 174) gives as examples *Fomes noxius* on *Tephrosia* and *Grevillea,* and *Ustulina zonata* on tea and rubber.

GERMINATION

Water. Germination of spores is often dependent on the presence of fluid water, rather than on a water-saturated atmosphere. The uredospores of *Puccinia glumarum* (230), the conidia of *Peronospora destructor* (260), of *Pseudoperonospora humuli* (146), of *Colletotrichum trifolii* and of *Sclerotinia fructicola* (256) need actual films of water for germination. Others, like *Cladosporium fulvum,* may germinate in water and in a saturated atmosphere (81, 99, 113), but, as mentioned earlier, the conidia of *Sphaerotheca humuli* v. *fuliginea* germinate very badly or not at all in water or sugar solutions, whereas they do so readily at 100% humidity (91). Instances where very high humidity is essential for spore germination, but not free water, are *Sphaerotheca pannosa* v. *rosae* (139), which fails to germinate at 75% humidity, and *Podosphaera leucotricha* (14). Conidia of *Erysiphe graminis* can germinate in a completely dry atmosphere (29), and this is also true for those of *E. polygoni* (20, 256). Whether the conidia of the latter fungus germinated or not, those produced at the lowest humidities soon shrivelled and died, while those produced at 80% were merely smaller, though not so turgid, as those produced at 100% humidity. Also, the growth of the mycelium was reduced by low humidities (256). While these conidia remain attached to the conidiophore there is no mechanism for the passage of oxygen and therefore presumably of water, for it has been demonstrated that the papilla by which the conidium is attached to the conidiophore is the only place where such passage is possible, and this is exposed only after detachment (20).

Temperature. The temperature factor is very intimately associated with germination, but, apart from a few special points, it is not proposed to list details of temperature ranges for the different fungi (see 59, 81, 82, 113, 146, 180, 193, 230, 231, 260). It has already been mentioned that low temperatures may harden spores to extreme exposure; similarly, there is a process of temporarily warming spores to increase their germinative capacity. The uredospores of *Phragmidium mucronatum* germinate better if exposed to 27° C. within eight hours of sowing the spores for a germination test. Held for one hour only at the optimum temperature for germination, 18° C., the spores germinate better when removed to other temperatures (36). It is considered that brief spells of favourable temperatures in the field may induce spore germination on leaves, even when the weather is generally unfavourable and caution is required in applying results of laboratory tests to field problems (36). Sclerotia of ergot (*Claviceps purpurea*) germinate better if they are frozen before placing them at 9°–15° C. Temperatures of 18° C. and above are inhibitive to the germination of these sclerotia but favour perithecial development (130). This fits in with the advance of the season and the development of the fungus. Any unusual capacity of spores to germinate at high temperatures always leads to the danger that a disease when introduced into a fresh area, even though possessing a hotter climate, may readily become established. This danger has been foreseen in the case of *Cronartium ribicola,* 31% of the aecidiospores of which germinate at 28° C. (100).

Microclimate. Some useful information has been published on the microclimate of leaves in relation to disease. Moving air not only directly affects the percentage of relative air humidity but also affects the germination of spores by introducing another undetermined factor. Conidia of *Podosphaera leucotricha* germinate and infect apple leaves only at 100% humidity if the air is moving (43 feet per hour), even a drop to 93% humidity inhibiting germination. In still air germination takes place down to 34% humidity (227). It is possible that in still air gaseous and other excretions from the leaves stimulate germination in spite of less favourable moisture conditions, as it is well known that host extracts stimulate germination of conidia of *Pseudoperonospora humuli* (146) and *Sphaerotheca pannosa* v. *rosae* (262), amongst others. Longrée claims (139) that with the last mentioned fungus there was a very high humidity on the leaf surface, even though the general atmosphere was dry, and that this accounted for activity of the fungus. It is suggested (262) that the factor which favours conidial germination when atmospheric humidity is low, is host stimulation. Further, the temperature of the leaf surface is lower than that of the surrounding air and leads to condensation of moisture on the leaf, thus favouring spore germination (258).

Nutrition. Another complication is that temperature ranges may be altered by nutrition. Phosphates raise the maximum temperature for germination of uredospores of *Puccinia glumarum* by 2°–3° C., while peptone and asparagin do so to a less extent. Carbonic acid gas, at 3% and 6% concentrations, reduces and retards germination at temperatures below 12° C. but increases the velocity and extent of germination at 17°–19° C. (230). It has been claimed that an exact knowledge of spore germination temperatures may be of direct use in control experiments. Control of *Sclerotinia fructicola* by sulphur dusting is stated to be better at 40°–55° F. than at 65°–85° F. which is the optimum range for spore germination (141).

DISEASE INITIATION AND EPIDEMICS

Infection. The effect of the various environmental factors on actual infection has also been studied. Several species of *Erysiphe* (*polygoni, graminis, cichoracearum*) are able to infect their hosts at low relative humidities (25%–55%) (256). With *Sphaerotheca humuli* v. *fuliginea* infection is best at 69% to 96% humidity (91). Infection of lettuce leaves by *Bremia lactucae* takes place only at 100% humidity (181, 209), while that of rice by *Helminthosporium oryzae* cannot take place under 80% humidity (134). Even though germination of the uredospores of cereal rusts may be rapid at low temperatures, the infection process may be much delayed at those temperatures (230). This is confirmed specifically for *Puccinia coronata,* for the development of which and the production of uredosori the plants must be post-incubated at 20° C., even though infection can take place at 0°–2° C. (59). A precise analysis of the various stages involved in infection by *Puccinia triticina* demonstrates the fact that optimum conditions must last for some time to permit critical development of the disease. At 23° C. uredospores germinate in one hour, appressoria are found in three to nine hours (two to eight hours after germination), penetration tubes one hour later. In 24 hours almost all spores form substomatal vesicles and three-quarters have infection hyphae. The lower temperatures delay the initial processes, and once these have taken place it appears that the infection hyphae develop inside the hosts rapidly enough to catch up with the stage of development at higher temperatures. The higher humidities favoured maximum amount of infection (7).

The importance of standardising temperature and other factors when testing wheat varieties and strains of *Puccinia triticina* has been stressed. Apparently, some varieties become more resistant at 6° C. instead of more susceptible, and others in a saturated atmosphere become more susceptible instead of showing the usual increase in resistance (92). Such aberrant varieties lose their resistance when deficient in nitrogen. When conducting susceptibility tests on potato tubers for their blight reaction, it is essential to maintain correct temperatures, that is, temperatures at which the host reaction to invasion (by development of necrotic areas) is at the optimum, namely, 16.5° to 21° C. (166). Another way in which temperature acts is by masking symptoms of actual infection, which is different from suppressing infection. High temperatures of

32° C. over a long period mask the symptoms of *Ustilago striaeformis* on *Poa pratensis*, but the symptoms return on reducing the temperature to 20°–25° C. (131).

Although the physiology of parasitism is outside the scope of this review, a few points should be mentioned, as determinations of temperature, moisture and nutrition may be involved. For example, those diseases which rupture the epidermis, such as rusts, gall-forming diseases and scabs, lead to an increased rate of transpiration, whereas the downy mildews cause only a slight increase. Powdery mildews may, on the other hand, even reduce transpiration by adding protection to the cuticle (98). Respiration rates, independent of those of the parasite, also increase with infection by a powdery mildew like *Erysiphe graminis,* though the rate may decrease after a time (2, 3, 189). Teleutospore formation of rusts is now known not to be initiated by nitrogen exhaustion of the leaves, and the new theory is that the stimulus is the gradual loss of water from rust-infected leaves, accompanied by a rise of nitrogen content. The uredospore stage is not stimulated by a loss of water but is favoured by high humidities (70, 206). High root pressure might well be expected to affect the susceptibility to, and progress of, bacterial diseases (117), but high osmotic values in the parts of maize plants infected with *Ustilago zeae* are associated with the higher degrees of infection (192). The correlation is supposed to be associated with the more luxurious growth of plants having high osmotic pressures. However, wheat varieties with high osmotic pressures were reported as more resistant to *Puccinia triticina,* as also were varieties with small narrow leaves (206); hence, there is no consistent pattern of behaviour in this respect, each disease being specific.

Epidemics generally. Gaumann has summed up the principles of epidemiology thus: It is necessary to have (*a*) large populations of the susceptible host, (*b*) a high epidemiological potential of the pathogen, and (*c*) optimum conditions of weather for the pathogen (71). He might have added, however, a fourth condition, namely, that it is necessary to have conditions which suppress any tendency towards host resistance.

The brown rust of wheat caused by *Puccinia triticina* depends first on survival of sufficient inoculum into the winter period. Hot summer weather may have such a deleterious effect on uredospore germination, as happened in 1931 and 1934 in Sicily, that no epidemic follows in the next year, in spite of otherwise favourable weather (163). Granted this over-summering of uredospores, Chesters (31) concludes from an analysis of 16 severe epidemics and 17 mild epidemics of this rust that the intensity and virulence of an epidemic in any year are governed by the temperature and rainfall in the period of December to March (April in some areas). He finds that the April–May and the June conditions have little effect. Since temperature and moisture are so near the threshold permitting rust multiplication in the winter period, quite minor fluctuations in these conditions turn the balance favourably or otherwise. When these favourable conditions do exist, the rust increases in the winter by a series of uredo generations. In the spring, fluctuations of these conditions are always within the range favourable to the rust. The period of incubation, of course, may fluctuate according to spring conditions, and forecasts of this period may be made (171), as may also be done for *P. coronifera.* Forecasts were made of epidemics of brown rust in Oklahoma in 1945 and were based on the amount of inoculum (17,000 times as much as in 1944) combined with high temperature records for 23 out of 24 days in March (32). Similar correlations have been found in other areas (147, 156), though in Canada the critical months are later (April–May and July) (41).

Black rust (*P. graminis tritici*) similarly depends on the early season temperature and moisture records, the minimum temperature being the more important, as it controls uredospore germination, prevention of which may delay an epidemic for months or suppress it; the maximum temperature is of secondary importance and the mean of no significance. Rainfall and dews in early spring and in midsummer, when the disease is spreading, are of vital importance (41, 121, 147, 156). Severe epidemics of this rust in the U.S.A. in 1916 associated with warm wet weather in July (108), in Kansas in 1935, 1937, 1938 and 1940 (121, 126, 153) and in Texas in 1935 (8) caused considerable loss and were traceable to temperature and moisture records which favoured initial outbreak and spread.

In the north Caucasus all three wheat rusts (*P. graminis, P. glumarum, P. triticina*) were found to be epidemic with considerable loss of crop, since the climate permitted the overwintering of these rusts, and the abundant rainfall and dew in May and June, coupled with the high day temperatures of June, facilitated infection and spread (12). Mehta and his colleagues (148–150) have shown that the main source of infection of the principle wheat areas in the plains of India are uredospores blown down from the hill crops, since the teleutospores are scarce, being formed in, or subsequently exposed to, the very high lethal temperatures of April–June. Such infection of barberries as takes place is too late to serve as a source for infecting the commercial crops in the plains. Control therefore consists in suspending cultivation of hill crops for two to three years, until resistant crops are developed, or in destroying self-sown plants and stubble some time before sowing the seed (if this has to be done at all in the hill areas), or in suspending the first crop in April in the Nilgiris and other hill areas and postponing until October the sowing of the main crops in central Nepal.

Little has been said so far about physiological races. Since evidence is accumulating to show that strains or physiological races differ in their relation to environmental conditions, it is imperative to know what effects may be expected when once the strains of any fungus in a given district have been identified, for only then may the danger of epidemics be assessed in relation to the weather which may normally be expected. For example, strains of *Puccinia anomala* vary in relation to temperature, some being less, some being more, virulent at 12° C. than at 22° C. (196). Generally, temperatures higher than the optimum may reduce the vigour of uredosori development, progressively passing with increase of temperature from No. 4 type (very susceptible) to No. 3, to No. 2, to No. 1 and to No. 0 (necrotic flecks) type (118). In addition, varieties of cereals may be grouped according to their degree of fixation to infection types. Three strains of *P. graminis tritici* have been tested on 18 wheat varieties in Canada and found to fall into three groups: (*a*) immunity retained with any condition, (*b*) immunity lost but moderate resistance retained at high temperatures of 80° F., and (*c*) those which become susceptible at the high temperatures but are immune or moderately resistant at low temperatures (119). The importance of this was demonstrated by the fact that the "Kenya" and "McMurachy" varieties were less resistant in the higher temperatures at Minnesota than at Winnipeg. Strains of yellow rust (*P. glumarum*) in Germany have been tested similarly on numerous wheat varieties, but a classification of varietal reactions was made which is different from that above worked out for black rust, thus: (*a*) summer resistance, increasing with higher temperatures and advancing age, (*b*) susceptible at any time or age, and (*c*) resistant at any temperature above 10° C. The (*a*) group is virtually immune in the growing season but bears abundant pustules in autumn and winter (219). However, susceptibility may be increased by nitrogen starvation or infection with bunt.

Other rust epidemics have also been studied and correlated with weather conditions. Spread of *Cronartium ribicola* was considerable in several States in U.S.A. in 1941 and was associated with warm, wet and cloudy weather. The disease was spread 134 miles by wind from West Virginia to North Carolina (185). A further analysis of the necessary weather conditions was made by Hirt who showed, however, that the general seasonal conditions were not a reliable index to the amount of this rust that might be expected. Each year when epidemics of this rust occurred in U.S.A. the bulk of the infection took place in one or two relatively brief periods of cool, wet weather at a time when abundant freshly produced teleutospores were available. Generally, for infection of pines the most important factor was the abundance and length of duration of rain, while for sporidial production either rain or fog was adequate (101).

Concerning potato blight, most workers confirm Everdingen's rules for predicting outbreaks of this disease in Holland (241), in Argentina (52, 75) and U.S.S.R. (170), but outbreaks in South Carolina are claimed to be less dependent on mean temperature and relative humidity than on the amount and distribution of rainfall in the growing season. It is claimed that epidemics can be predicted according to the early season's rainfall (164). Beaumont, however, claims that in Devon the two days' high humidity rule is sufficient to forecast outbreaks of blight, though high temperature is also necessary (10, 11). It has been possible to map out areas favourable and unfavourable to blight epidemics covering the period mid-August to mid-September, and these were correlated with actual outbreaks in the U.S.A. (4). The importance of chilling is shown to be related to the germination of the sporangia formed in the previous high humidity which must persist for 12 hours to permit infection (42). In Colorado, where normally the hot dry summers are unfavourable to blight, the occurrence of fog in 1941 and 1942 coupled with the previous heavy winter snows (which facilitated survival of infected tubers) led to outbreaks of blight (207).

The source of blight inoculum has long been debated, and Limasset in France claims that infected tubers may provide that inoculum, the conidia of which may be able to survive the particular micro-climate round that plant, even though the general weather is unfavourable. He points out the importance of distinguishing between susceptibility and receptivity. For example, "Early Rose" is a potato variety which is as susceptible to blight as the variety "Saucisse", but it is much less receptive, as its aerial parts are not luxurious enough to provide the favourable micro-climate (135). In the U.S.S.R., also, Naoumova has found that blight survives one season in the buried infected tuber debris. When such debris is brought to the surface the fungus in it sporulates and serves as a source of inoculum for fresh outbreaks. Diseased leaves and tubers kept dry overwinter were used successfully to infect potato stems, but other material left to overwinter naturally in the field failed to infect healthy potatoes. In spite of this, it is claimed that natural overwintering is possible under some as yet unknown conditions of temperature and moisture (173). Bonde and Schultz, however, have shown experimentally that while infected seed potatoes left in the field over winter may give rise to infected shoots, the latter do not serve as important sources of infection for epidemics. They traced the source of a natural epidemic to a refuse-pile on which blight had developed before spraying had commenced, and they showed that the percentage of infection of the crop decreased with increasing distance from the refuse-pile. They also created an artificial refuse-pile and traced the spread of blight from it with the following results:

Distance from refuse:	100 ft.	200 ft.	300 ft.	400 ft.	500 ft.	600 ft.
Percentage blight:	98%	55%	21%	6%	0%	1%

Similar results were obtained in later experiments (15). Literature on similar correlations of weather with various diseases is very extensive, but only representative papers are cited in the bibliography of this article (18, 19, 55, 82, 83, 97, 102, 110, 126, 137, 154, 158, 168, 210, 213, 226, 252, 267).

SOIL CONDITIONS AND PLANT DISEASES

Garrett (66, 67, 69) has brought together the literature on this subject and has analysed the factors involved in the survival and activity of soil-inhabiting plant parasitic organisms and the diseases they cause. Much of the older literature reviewed by him has been reviewed elsewhere (57, 58). He considers that soil conditions will exert the greatest direct influence upon the organisms which spread in the soil external to the roots of their hosts but indirectly through the physiology of the host upon those organisms which pass their life largely inside the host. He considers that survival of a fungus in the soil is conditioned more by the increase or decrease in the amount of inoculum left in the soil than by direct effect on a given quantity of inoculum. For example, *Phymatotrichum omnivorum* may be left in larger bulk after harvest in neutral or acid soils, and survival of *Ophiobolus graminis* may be favoured by a set of soil conditions unfavourable to its parasitic activity. He points out the influence of competitive microflora on any given pathogen: both *Ophiobolus graminis* and *Helminthosporium sativum* have the same optimum temperature of 25° C. in culture, but in the soil the optima are, respectively, 18° C. and 25° C., shown to be due to masking of temperature effect by antagonism of microorganisms requiring high soil moisture, such as club root, powdery scab, wart disease, various species of *Phytophthora* and allied organisms, the life histories of which necessitate ample supplies of water; also some *Fusarium* diseases, *Helminthosporium sativum, etc.* The diseases which are favoured by wet seasons but the development of which is independent of high soil moisture and which are stopped by unusually dry seasons, are those caused by *Phymatotrichum omnivorum* on cotton, limited by the amount of rainfall from April to July, and *Ophiobolus graminis* on cereals, limited by weather in the spring months (June–August in Canada, August–October in Australia). The diseases favoured by low soil moisture are potato common scab, most smuts, *Gibberella saubinetii* and some *Fusarium* diseases.

Texture of soil has a considerable influence on soil-borne diseases, and while a few diseases are favoured by heavy soils, most are favoured by light soils. In light soils, both high and low moisture groups of diseases exist, though many of the diseases are favoured by low moisture conditions. This is correlated with the aeration factor which, however, Garrett considers should not be overestimated, since many diseases are equally affected by soil reaction, itself frequently, but not always, associated with soil texture.

It is very natural that fungi such as *Phytophthora* and *Pythium* should depend so largely on high soil moisture, since the liberation, and especially the spread, of zoospores depends on a sufficiency of water. *Pythium irregulare* attacks and kills red pine seedlings most rapidly at high temperatures and also reasonably quickly at 12° C., but it requires water-saturated soil. The optimum conditions are thus wetness, high temperatures and not too acid a soil (198, 199). *Pythium ultimum,* however, can be very destructive even at 60% soil moisture, provided the temperature is at the optimum of 18°–21° C. (5). *Phytophthora cactorum,* while it needs ample water, also requires aeration, since diseases caused by it are optimum at 96% soil moisture (249). Strains of this latter fungus vary in their temperature ranges, but the moisture factor remains constant; the same water relations hold for *P. parasitica* (236, 250).

Garrett placed many smuts in the low soil moisture group; in addition to his own list *Urocystis occulta* on rye (138) and smut of sorghum (155) could be included. The sclerotia of *Sclerotinia sclerotiorum* have been killed by flooding the soil with water (21), yet high soil moisture is said to favour this fungus, and one can only presume that in this case the aeration factor mentioned by Garrett had a large part to play. These sclerotia are known to survive in dry soil for 11 years. Usually *Actinomyces scabies* is considered a dry-soil organism, but Sanford (204) has found it to be as active in moderately wet soil (39% moisture) as in dry soil (19%). This organism grows well in soil too dry for plant growth (204). None of this cancels the conception that moist, but not wet, down to dry soil, with warm temperatures about 20° C., conduce to severe scab when tubers are growing actively (see 57). That tubers of high vitality are more readily infected has been shown experimentally to be correct in Switzerland (72). Four Egyptian smuts, all recognised as low soil-moisture diseases, are there controlled by planting the host seed in very moist soil, at a shallow depth, and the practice works well where irrigation is carried out. Where the soil surface dries rapidly, the seeds may not penetrate the hard soil on germination; hence, this difficulty is overcome by first soaking the seed, then coating them with mud and then planting (122, 123).

Kramer has provided a survey of the physics of soil moisture which may alter some conceptions on interpretations of the relationship of soil moisture to plant diseases. Gravitational moisture, while it provides the source of water for growth, is not directly related to the ultimate growth rates of plants. What Kramer calls the "field capacity" is of more value in this relation to growth rates, as it approximates the "moisture equivalent" of the soil. The variation in soil type from sand to clay, with various percentages of organic contents, affects the field capacity. The water which lies between the field capacity and the "permanent wilting percentage" is that which is available for plant growth. The nearer the water content of the soil gets to the permanent wilting percentage, the more rapidly does the water become less available. Capillary water movement in the soil below field capacity and above the level of the water table is very slow, and it is supposed that roots obtain water more by growing into contact with the water than by water reaching the roots by its own movement. Little or no water is lost by evaporation below eight to twelve inches depth. One of the most significant claims is that it is impossible to half wet a soil and that it appears practically impossible to maintain intermediate moistures. That is, a part of the soil will be wetter to full field capacity and the rest will be unaffected (129). If we read for "plant" or "root" in the above the word "fungus", it will be realised that such a new conception may explain some of the discrepancies which have occurred in experimental work; it particularly may have reference to field conditions rather than to pot and soil moisture control tanks. In any case the plant pathologist may feel constrained to re-examine experimental results in the light of such information as given by Kramer.

The relation of environmental factors with *Ophiobolus graminis* has been poorly understood until recently, and Garrett gives summaries of the results of experiments by himself, Winter, Fellowes and others up to 1944. It would seem that the disease is severe and widespread in light textured and loose badly compacted soils. Thus a poor seed-bed resulting from too late and hasty preparation, ploughing in of long straw stubble and dry grass, dry ploughing and incorporation of unrotted stable manure, all favour the disease. Wet springs and summers are correlated with the disease, but too abundant moisture is unfavourable and may be related to reduction of aeration. Any relation of oxygen and carbon-dioxide to the activity of the fungus must concern the rhizosphere, that is, the very immediate neighbourhood of the root system, since conditions in the general soil atmosphere are different from the microclimate of the rhizosphere, owing to root respiration and microbial activity of the latter. (Special papers are 54, 69, 255). Other instances of the different temperature relations of various soil diseases are given in other papers (76, 155, 219, 264).

APPARATUS

An American committee on apparatus used in aerobiology surveyed in 1940 the history of air analysis, for microbial population in relation to human and plant pest and disease epidemics (39). Details were given in their report on the more outstanding historical techniques such as Rettger's Air Washer (1910), Owen's Dust Impinger (1922), Stakman's Paddle and Bottle (1923), Well's Air Centrifuge (1933), Well's Impinging Centrifuge (1937), Schmidt's trap, Lindbergh–Meier Sky Hook (1935) and the adaptations devised by Proctor (190, 191), Parker, Christoff, Mehta, Chatterjee and others. A survey also was made of the work and results of the pioneers in air-borne analysis, wind distribution of spores, rates of fall of spores, much of which has been previously, and also here, reviewed. Another recent apparatus is Krogh's microclimate recorder (132) which combines the essential features of a pocket thermohygrograph with the usual meteorological micro-recorder. Apparatus for testing plants for their response to controlled environmental factors, such as temperature, soil moisture, humidity and light, has consisted mainly of adaptations of the basic chambers built by Jones and his colleagues, aiming at saving space and cost. Improvements in fineness of control, in more even distribution of temperature and humidity, and in provision of adequate ventilation have also followed, many of which have not necessarily been described in publications (26, 106, 237, 246, 251).

PRACTICAL APPLICATIONS

Several examples have been given in the preceding pages where knowledge of the environmental effects on fungi or diseases has been utilised to control these diseases either directly or by breeding methods, and further examples may be mentioned here. As reported earlier (58), several countries were fast developing before 1935 spray-warning services with special reference to potato blight, vine mildew and apple scab. The present position of the European continent is not known, but at least some research had continued up to 1943 to determine the date of discharge of ascospores of *Venturia inaequalis*. For example, scabbed apple leaves collected at regular intervals and kept under damp conditions in the laboratory discharged spores a few days before discharge occurred in the orchard; hence, provided a regular collection of overwintered leaves was made, the first date of discharge in the laboratory could safely be used to predict when outside conditions would favour initiation of a scab epidemic (103, 104, 105, 116). Prediction of suitable spraying dates for the control of downy mildew of the vine has received more attention in Europe than any other disease. Recent work has demonstrated that a set of rules drawn up for one district for this purpose could not be applied to all areas with equal success; Müller's formula, successful in Germany, is unsatisfactory in southwest France (208), and the Italian oil-spot method was also unreliable in France. In Italy four superfluous sprays could be obviated by one well-timed spray, made possible by accurate forecasts of the outbreak of this disease (200). It is of interest to note the methods of prediction used in France. The primary outbreak can be estimated by the rainfall in the period November to April and by the stage of host growth and oospore germination of *Peronospora viticola* in spring, while the secondary infections can be determined by the condition and number of the primary infections, the relative humidity, period of rainfall and temperature records before, during and after rainfall (208).

As regards forecasting potato blight epidemics in Great Britain, it was found that Beaumont's five rules could be reduced to two, namely, a minimum temperature of 50° F. or over and a relative humidity of 75% for at least two days. With these two recordings forecasts of blight outbreaks were as satisfactory as when using the previous five rules (10, 11). No practical application of this method for forecasting blight has been practised in Britain, however. In the U.S.A., owing to the large losses caused by this disease at a time when food was of primary importance (25 million bushels of tubers were lost in the Upper Mississippi area alone), a forecasting and spray-warning system was initiated in 1943, reports being sent in by recorders in ten Federal States and two Canadian Provinces. Apparently, rainfall and temperature records were considered sufficient: 70° F. was the maximum temperature conducive to optimum development of the causal fungus, and in no case was blight virulent where the mean monthly temperatures in June and July were above this figure (157). There is no published evidence that a forecasting system has been used for downy mildew of tobacco (*Peronospora tabacina*), a disease which is important in many countries. In the U.S.A. it is suggested that such a scheme would be both possible and useful, since it is claimed that when the mean January temperature approaches 62° F., the mildew will develop, provided the conditions in early summer are also conducive to infection (162).

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THE BOTANICAL REVIEW

VOL. XII DECEMBER, 1946 No. 10

DESTRUCTIVE PLANT DISEASES NOT YET ESTABLISHED IN NORTH AMERICA

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INTRODUCTION

The welfare of our plants is a matter of vital concern to all of us, for without plants we would soon be without food, clothing and shelter. As befits the importance of the subject, there is a voluminous literature on various phases of plant welfare. From time to time some of the problems involved in the occurrence, development and spread of plant diseases have been discussed. But despite their potential importance, the status of destructive foreign plant diseases, in general, as a part of the plant welfare problem, seems to have received relatively minor attention (22, 72, 96, 106, 107, 125, 128). The continued appearance of serious new diseases in foreign countries, and of new information regarding old foreign diseases; an increased recognition of the seriousness of the problem of foreign virus diseases; the finding of biologically distinct foreign strains of many pathogens; the prospect of increased importations of foreign diseases with newly gathered plant products transported by high-speed aircraft; an increasing knowledge of the possible influence of trace elements, antibiotic substances, bacteriophages, hormones and so forth on the incidence and severity of plant diseases; and the possibility of new methods for eliminating some of the risks inherent in plant importations—these changes, and impending or possible changes in conditions, make it desirable to review the status of destructive foreign diseases with greater frequency.

Destructive plant diseases introduced from abroad are costing us millions of dollars a year for control, millions of man-hours in the production of the hosts whose usefulness they destroy, and incalculable losses in economic and other adjustments and in lost beauty in landscapes and gardens. Other diseases not known to be established on the North American mainland appear to be capable of causing great additional destruction. Some of our introduced diseases, such as citrus canker (72) and chestnut blight (63), were unknown until they began destroying plants here. Others, such as white pine blister rust (72) and Dutch elm disease (85), were known to be destructive abroad before they reached us. The powdery scab of potato was so serious elsewhere that its introduction was greatly feared, until it was found to be well established here and usually unimportant. Some of the factors affecting destructiveness will be discussed later. They are sufficiently complicated to make it impossible, on the basis of present knowledge, to predict with certainty what will be the future status here of specific foreign diseases that may be introduced.

Our information regarding even the older destructive foreign diseases, and the pathogens causing them, is entirely inadequate in many essential details to enable us to prepare complete defenses against them effectively and economically. Such data as have been published regarding foreign diseases are to be found in thousands of books and articles, frequently in difficult foreign languages, or in obscure or little-known publications. No attempt is made in this review to list or even to summarize these individual items as such. Instead, a few dozen diseases were selected, and this paper is based primarily on data regarding them. While our knowledge of these diseases is far from complete, the examples selected illustrate the complex factors which must be taken into account in evaluating the status of foreign plant diseases. The pathogens, or causal agents, of the diseases selected include various kinds of fungi, bacteria, viruses and nematodes, a diversity of methods of reproduction, survival and spread, and an equal diversity of ecological conditions favorable to their development. These pathogens have different hosts, including plants used for food, feed, forage, fiber, timber, and ornamental or other purposes. These pathogens may affect their hosts by causing root rots, stem rots, cankers, leaf spots, fruit spots and rots, disfiguring diseases, sterility and wilts, or having other more general effects. Furthermore, the examples selected illustrate the fact that all foreign continents and many islands harbor destructive plant diseases not yet established on this continent.

A disease may be considered destructive if it causes small but widespread losses on a major crop, such as wheat, corn or potatoes, or if it causes severe losses of useful plants of any kind. Any disease may be considered to be established if the available information indicates that it is here to stay, but not if it is being eradicated, as are citrus canker and potato wart.

EVALUATING DESTRUCTIVENESS OF PLANT DISEASES

There is no standard method of evaluating the destructiveness of plant pathogens. This is readily understandable when the variations in hosts, diseases and circumstances are considered. We speak of destructiveness from the point of view of man, which is not necessarily that of the host. Potted foliage plants might be disfigured and ruined for sale purposes by a leaf spot even though the growth, vigor and reproduction of the host seemed unimpaired. For other plants, grown for fruits or seeds, such leaf spots might be unimportant. Approximately two-thirds of the pathogens listed in this paper affect leaves, and about the same proportion affect stems of their hosts.

In some cases, such as the leaf and stem diseases of grasses[1], lespedeza, sugarcane and hemp, the leaves or stems may be the important crop part of the plant; but in others, such as flag smut of wheat, maize streak and pea rust, the seed is the important crop part, and for beet curly top, the rust of carrot and sweetpotato scab, the fleshy roots or tubers are the crop part of the host.

In tristeza and mal secco of citrus, court-noué of grape, smut of *Brassica* spp., root rot of asparagus and *Vialina* on pyrethrum the roots or lower part of the stem become affected. In three of these plants the crop is fruits; in one it is roots, leaves or seeds, depending on the host species involved; in another it is young stems; and in the last it is flower parts and stems. In potatoes the crop is tubers. Wart and buba disease ruin potato tubers, but the virus diseases and golden nematode may reduce the yield by weakening the plant rather than by destroying the tubers. When a plant is weakened, as well as when the salable part is blemished, the quality or grade as well as the volume of the crop may be reduced. Thus, reduction in yield (including quality) of the crop part of a plant is frequently a secondary effect of the pathogen, and estimates of the reduction are subject to error, but estimated yield reduction seems to be the best measure of the destructiveness of most pathogens.

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[1] See section, Examples of Destructive Foreign Diseases.

There are, however, other losses to consider in any discussion of destructiveness of plant pathogens. The beauty of a landscape may be changed or ruined with little change in real-estate values even though the esthetic loss appears to be heavy. If a crop is destroyed early in the season, the ground may be replanted and the net loss greatly reduced. A partial crop may bring such high prices that crop loss is largely or wholly compensated for, insofar as net cash return is concerned.

Many pathogens and pests, as well as adverse growing conditions, may be inextricably combined in causing losses. Loss of ground cover may increase soil erosion, increase flood damage and affect power production with resulting disruption of industry. Crop losses which cause shortages of raw materials for industry, resulting in lost jobs or reduced earnings and consequent lowered standards of living, are losses difficult to evaluate. Crop losses which increase the cost of living of industrial workers are important whether the grower loses money or not. Likewise, crop losses, actual or threatened, which force the grower to work overly long hours to protect his crop, maintain his yield and make ends meet cannot be evaluated readily. In some crops the margin of profit is so small for many growers that an additional relatively small percentage loss might be disastrous.

Despite the impossibility of evaluating either direct or indirect losses accurately, the fact remains that plant diseases, new or old, sometimes cause heavy losses and force economic readjustments which are painful to many people.

FACTORS AFFECTING THE DESTRUCTIVENESS OF PATHOGENS

Some pathogens are more destructive than others, and the destructiveness of a given pathogen varies with conditions. Disease incidence is usually dependent on the interaction of several factors, and the outcome of the interaction is not readily predictable, even where records of the results of more or less similar combinations in the past are available for the same area. It is obvious, therefore, that we are in no position to predict with accuracy what will be the action of each specific destructive foreign disease which may be introduced in the future. However, if suitable data are obtained from foreign areas where those diseases occur, predictions based on such data will be approximately correct much of the time. Some of the factors that affect the destructiveness of plant pathogens are mentioned in the notes that follow.

Virulence of pathogen and susceptibility of host. The destructiveness of a pathogen depends, first, on its virulence, which may vary in different individuals or strains of the pathogen, and second, on the relative susceptibility or resistance of the host, which varies with species, varieties and individuals, and with age and other conditions. "Resistance" is used here as a general term to include any chemical, physical or other characteristic which hinders infection by, or development of, the pathogen.

Few pathogens, either foreign or domestic, act with the swift destructiveness of common downy mildews, such as *Pseudoperonospora cubensis* (Berk. and Curt.) Rostew. on cucurbits, or of potato late blight (*Phytophthora infestans* (Mont.) D By.), which may cause the sudden collapse of their hosts over considerable areas of a planting. Many pathogens, however, are destructive enough to eliminate possible profits by killing their hosts prematurely or by destroying or limiting their usefulness in some way. Orange trees affected by tristeza (155) may die in a few months after they become infected. In lemon trees infected with mal secco (110) the fungus grows upward at the rate of about two feet a month, soon killing the tree if infection takes place in the roots or lower part of the tree, but growth downward from infected twigs is very slow and death from such infections does not result so quickly. In carnations *Verticillium cinerescens*[2] (160) was found to have a mean rate of growth of approximately 0.6 cm. per day during June and July, and 0.4 cm. during September and October. The virus causing pupation disease of oats (139) is able to spread through host tissues at the rate of 7 cm. per hour, fast enough to invade an entire plant in a quarter of a day or less, but the host is stunted and made abortive, rather than killed as soon as invasion of the tissue is completed. The rate of movement of the maize streak virus (132) varies, but is said at times to exceed 20 cm. per hour down infected leaves.

Cronartium flaccidum rust (15) of hard pines stunts and ruins affected hosts but does not necessarily kill affected tissue; in fact, the affected tissue is rather fully invaded for some time before the fungus is ready to produce spores. Maize streak (133) is ruinous on infected seedlings but does little damage to plants not infected until nearly mature.

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[2] Authors for binomials listed in the section Examples of Destructive Foreign Diseases are not given elsewhere.

Variations in virulence of pathogens have been studied in rusts and smuts, especially, and numerous fairly constant strains, races or biotypes have been differentiated. New strains of various pathogens frequently arise through interbreeding of closely related forms or through mutation. These strains vary greatly in their virulence on a given host. Closely related host varieties also vary greatly in their susceptibility to infection and in the degree of injury induced by infection with a given strain of a pathogen. One would expect a pathogen or strain which is extremely virulent on a host to destroy it, as the chestnut blight (63) seems to be eliminating American chestnuts, but that does not necessarily follow. If a few individuals with some resistance are able to reproduce, and such of their offspring as are resistant continue to reproduce for a number of generations, the time may come when susceptible hosts no longer appear, and the pathogen seems to be nonvirulent. If this pathogen is taken to a new locality, or if a new supply of susceptible individuals is brought in, it may demonstrate its virulence again. Virulence of a pathogen to a given host is sometimes modified by its growth for a time in another host. Some strains of *Glomerella cingulata* (Ston.) Spauld. and Schrenk and of *Ditylenchus dipsaci* (Kuhn) Filip. will readily attack a wide variety of hosts. Other strains, long confined to a single host or a small group of related hosts, seldom invade other species. Peanut rosette (122) has been transmitted to *Centrosema plumieri,* but attempts to transfer it back to peanut have failed.

Some of the leafhoppers which transmit rice dwarf (46) must feed on diseased hosts much longer than others in order to be infective, and some do not become infective at all regardless of the time of feeding. The feeding periods required to make infective the individual vectors of the pupation disease of oats (139) are extremely variable also, the minimum being six hours, and never do more than 37% of the insects present become infective, even though all the oat plants are diseased. Vectors of maize streak (134) vary greatly in their ability to transmit the disease, and some races or groups of the insect species involved are not vectors, although some are able to act as vectors if the virus is artificially injected into them. The causes of variations in the ability of individuals to transmit virus diseases have not yet been determined.

Host plants transferred to new areas may be severely attacked by diseases of related plants in the new home. This happened to corn sent to the Orient, where the downy mildew of sugarcane and other related plants attacked it. However, if corn varieties developed in the Orient for resistance to downy mildews were brought back to this country, they might be found to have lost resistance to some of the destructive diseases here. Combining resistance to disease with quality and yield in new plant varieties is a major problem of breeders, and the frequency with which pathogens appear in the form of new races which are virulent on host varieties resistant to the old races makes it necessary to continue to develop new varieties, even if no new species of pathogens appear. New races of potato wart (18) in Germany are destructive on nearly all varieties developed for resistance or immunity to wart. New races of flax rust (148) in Argentina are destructive on new varieties bred for resistance to the rust forms in our flax areas. Races of flag smut in China (168) affect different groups of wheat varieties and might be severe on varieties resistant to any flag smut strain we have in this country. A large proportion of our important crop plants were introduced many years ago. Many diseases were introduced with these plants, but in the intervening years new races of the pathogens may have developed in the countries of origin, and selection and breeding may have led to radical changes in both host and pathogen in this country. Hence our choice varieties may succumb to common diseases if they are taken abroad, or if present-day strains of the pathogens are imported from abroad.

Absence of a pathogen or a race of a pathogen during one season is no assurance that it will be absent the next season. Asparagus root rot (156) reappeared after apparent absence for nearly 50 years and destroyed up to 80% of asparagus plantings in the type locality. The predominant strains of the much studied black stem rust of wheat have varied from year to year in unpredictable fashion. The rose wilt virus (54) may be destructive for several years and then appear sporadically for a year or two.

It is known that the vitamin content of plants used for human food varies with individual plants, with varieties, with species, with the soils in which the plants are grown and with conditions of growth. Similar variations of chemical compounds occurring in infinitesimally small amounts may account for some of the variations in the susceptibility of host plants, and in the virulence of pathogens growing on them. That is, lack of optimum amounts of vitamins or of trace elements or compounds in the plants may weaken their resistance to some diseases. Again, inability of the pathogen to obtain optimum amounts of some vitamins or trace elements from the host may weaken its parasitism (56, 117). It is known that bacteriophages (69), or virus diseases of pathogenic bacteria, occur. However, our knowledge of the effects of trace elements, of bacteriophages, of antibiotic substances produced by various hosts or organisms and lethal or inhibitory to some pathogens, of vitamins that may be needed by hosts and by pathogens, and of hormones affecting them, is too fragmentary to enable us to evaluate their rôles in regulating or determining the virulence, or apparent virulence, of destructive foreign pathogens or the susceptibility of hosts to such pathogens.

Reproductive capacity of pathogen. The reproductive capacity of the pathogen is another factor in determining its destructiveness. A pathogen which killed every host infected but which seldom produced a new infection might be unimportant and soon die out. Pathogens causing rusts, downy mildews and brown rots usually produce enormous numbers of asexual spores, often in several generations, during a single growing season. Smut fungi usually produce great numbers of spores, sometimes millions in a single mass, and each of these in turn may produce a number of sporidia—150 in a whorl in Karnal smut of wheat (99). Enormous numbers of bacteria are produced in infected hosts, as, for example in gumming disease of sugarcane (39, 122) and *Aplanobacter rathayi* (39, 122) on *Dactylis.* Such organisms, if distributed under favorable conditions, may cause so many new infections close together that infected leaves or stems will be killed very quickly. Viruses may increase very rapidly (8, 132, 139), destroying the usefulness of affected host plants, which at the same time become potential sources of infection. Multiplication of the virus in insect vectors is claimed in the case of rice dwarf (45), but there is a difference of opinion regarding the conclusiveness of the evidence (8). The troublesome nematodes may produce several generations a year and have numerous offspring each time. The black currant nematode reproduces throughout the year but most rapidly in the spring (97, 143). The nematode causing ufra disease of rice apparently does not multiply during the winter and spring, but even so, masses of new nematodes are produced on the host for three or more generations each year.

The amount of multiplication of a pathogen required to cause an epiphytotic is not measured by number or volume alone. Theoretically, a thousand spores may be able to infect and kill a thousand plants, but in practice the number of infected individuals per thousand host plants may be a hundred or fewer even, though billions of spores per plant are available. The spores must reach a large proportion of the hosts alive and under conditions favorable for infection, or there is no heavy infection, and the effective combination of all necessary factors just doesn't occur for most spores. However, the number of spores a fungus must produce to be destructive obviously depends on the effectiveness of its distribution and infection system. Air-borne spores, such as downy mildew or brown rot conidia and rust spores, which are scattered over large areas where infection is seldom possible, must be produced in vast numbers to insure even a moderate incidence of disease. Yet the perfect stage of downy mildews, produced in much smaller numbers but carried with the seed or left in the soil where the host will be planted again, may show a relatively high incidence of disease per billion spores produced.

Ability of pathogen to spread or to reach its host (22, 49, 62). Ability of a pathogen to spread, or to be spread, is a major factor in its destructiveness. Production of masses of spores or other forms of inoculum is not enough. The inoculum must reach numerous hosts by the aid of air currents, rain, insects or other animals, or on tools or farm machinery; or, in the case of pathogens carried over in the soil, hosts must be grown in the same soil again in order to cause epiphytotics. Spores of rusts (89), smuts (72, 99, 149), downy mildews (72, 159), and many others, as well as bacterial pathogens such as the one causing ash canker, are usually spread by air currents or splashing rain. The black currant nematode travels in water or is blown about when in a dry state (143). Dutch elm disease would probably be of relatively minor importance without the insect carriers of the pathogen. Many virus diseases, such as pupation disease of oats, rice dwarf, wallaby ear and maize streak of corn, are dependent almost entirely on insect vectors or carriers for their local spread. The incidence of pupation disease may be greatly reduced by providing barriers two meters high between oat fields and the fence rows, which are covered with grass and weeds on which the larvae of the vectors overwinter (138). The potato wart fungus is spread locally by water, and on tools and the feet of man or other animals. Many disease organisms are spread from place to place on a farm or to outside areas on seeds, plants or plant parts transported for food, feed or manufacturing purposes.

The spread of pathogens, both local and long-distance, is affected by continuity and size of plantings of the host. Stem rust of wheat starts in Mexico, and wheat plantings in the Mississippi Valley are so large and so nearly continuous that air currents sweeping the spores north cause new infections which supply additional spore crops and enable the rust to reach Canada by relays before the season is over. One-crop areas and pure stands of forest trees make it possible for pathogens, once started, to produce epiphytotics, even though their spore production and their individual distance of spread are rather limited. Of course, if wild hosts abound, pathogens may spread on them between scattered plantings of cultivated hosts. White pines may be well scattered and still succumb to white pine blister rust if aeciospores or urediospores are spread by air currents from distant points to ribes plants nearby. The chestnut-blight pathogen and other fungi produce such enormous numbers of wind-borne spores that isolated hosts may receive some of them and become infected. Insect carriers, birds, *etc.*, may aid pathogens in overcoming the theoretical advantage of isolation.

Sometimes there are special problems in the dissemination of pathogens. For example, the downy mildew (*Sclerospora sacchari*) affecting sugarcane and corn in Australia produces large numbers of short-lived conidia on corn but not on sugarcane; therefore the presence of corn plantings is necessary if the disease is to spread rapidly during the growing season. Gumming disease of sugarcane produces quantities of bacterial slime, but there seems to be little spread from diseased to healthy plants in the field, except where the soil is wet. Spread results largely from the use of infected canes for propagation purposes.

Court-noué of grape is spread by propagation from diseased plants. The same is true of potato viruses, tristeza disease of citrus, downy mildew of sugarcane, black currant nematode, and others.

While some pathogens, such as *Neovossia indica* of wheat, *Botrytis anthophila* of clover, *Pleospora calvescens* of poppy and soybean *Fusarium* disease, are carried in seed tissues, about a dozen others of those listed are carried as spores or otherwise on seeds or accompanying fragments of host tissue. Since such organisms are likely to be scattered through new plantings, only short distances from the initially diseased plants need to be bridged to bring about infection of all plants. Dodder plants have been found to transmit a virus disease from one host to another (11). Although it is not yet accepted as fact, there is evidence that crown gall is a virus disease of which *Bacterium tumefaciens* E. F. Sm. and Towns. is the vector instead of the causal agent itself (17, 56, 162, 163). If virus diseases are carried by bacteria and fungi to new hosts, some of the unimportant pathogens may take on a new importance. Virus diseases present a particularly difficult problem in prevention of disease spread, because there is no practicable method for detecting their presence in much of the plant material that is moved, even if the pathogen is present in abundance. Plants of little economic importance may appear to be disease-free throughout their lives and yet carry viruses destructive to important crop plants. Such masked carriers might be widely distributed and cause heavy losses without being suspected.

The probability of entry, establishment and spread of dangerous foreign diseases seems likely to be further augmented by increased use of aircraft in transporting freshly gathered fruits, vegetables and plants from all parts of the world. Incipient infections, viruses and adhering spores would be impossible to detect, by present inspection methods, on such materials at the time of arrival. By the proposed air routes, materials could be delivered to hundreds of scattered markets not far removed from our growing areas, instead of to a few big cities, mainly on the sea coast, where there is relatively little chance for a pathogen to survive. Mass movements, as a result of the war, have doubtless spread diseases into new areas. It seems certain that man's actions will combine to increase the likelihood of the introduction and spread of destructive foreign diseases, unless man devises and puts into effect improved counter measures.

Ability of pathogen to survive. A pathogen must be able to survive under unfavorable conditions if it is to be recurrently destructive. In mild climates, with hosts available at all times, a pathogen such as that of a rust or a downy mildew or a brown rot may persist indefinitely merely by producing short-lived summer spores. But over much of the world there is a cold season, or a dry season, or a host-free period, which must be bridged if a pathogen is to persist.

Many fungi, such as those causing potato wart, downy mildews and rusts, produce thick-walled spores able to retain viability through periods of cold or drought, or even for several years. Such resting spores of potato wart and downy mildew fungi may spend the off-season in the soil, sometimes remaining there for several years before the right combination of factors induces germination, or they may remain above ground in debris on the soil, or in storage places, and germinate later if given an opportunity. *Choanephoroidea* on squashes mummifies infected flowers and fruits, and these mummies doubtless serve as sources of infection the following season. Other fungi, such as the brown rot organisms, produce sclerotia that withstand unfavorable conditions. Still other fungi, such as the apple scab pathogen, may persist as saprophytes on dead leaves and in spring produce a perfect stage which starts new infections.

Many viruses and some of the rusts, smuts and downy mildews are systemic in perennial hosts; so if the host survives, the pathogen is likely to survive also. Some pathogens survive on or in hosts other than the one of particular economic value. The fungus causing shab disease of lavender, for example, can live through the winter as a saprophyte on dead goosefoot plants as well as on dead parts of lavender. *Cronartium flaccidum* rust of hard pines has numerous telial hosts, some of which are not of economic value. Many pathogens overwinter in seeds, among them being *Aplanobacter rathayi* of cocksfoot grass, *Botrytis anthophila* of clover, *Colletotrichum indicum* of cotton, and perhaps the mosaic of subterranean clover. Among the viruses that survive from season to season in insect vectors are those of pupation disease of oats (139) and of dwarf disease of rice (45). Some pathogens, such as those of common potato scab (*Actinomyces scabies* (Thaxt.) Güssow) and cotton root rot (*Phymatotricum omnivorum* (Shear) Dugg.), may live indefinitely as saprophytes in the soil and then attack hosts when they become available. Other pathogens, such as those of chestnut blight, white pine blister rust and Dutch elm disease, may remain viable and sporulate on host material after the host has been cut down.

It has been known for a number of years that some fungi are antagonistic to other fungi or to bacteria (47, 86, 111, 150, 151, 152). The most publicized cases of such antagonism are those involving penicillin, but there are numerous others. While considerable work is being done in this field, we have no specific data to indicate that any fungi or bacteria antagonistic to any of the destructive foreign pathogens discussed in this paper would aid in preventing their establishment here, but such a condition is quite possible. Even so versatile a creature as man failed in many of his early attempts to establish colonies in the New World. Introduced colonies of plant pathogens sometimes may find it just as difficult to contend with antagonistic native organisms and new conditions as did the early settlers with Indians and unfamiliar conditions.

Ecological conditions as related to host and pathogen (90, 164). Ecological conditions often determine the destructiveness of a pathogen. In general, hosts are grown where ecological conditions are favorable for their growth, and only such pathogens as are able to thrive under the same conditions will be destructive to the hosts. However, it is well known that some pathogens are especially destructive during a wet year, or following periods of rain or following early rains and mild temperatures or some more or less specific combination of ecological factors. Regulation of planting dates to avoid having seedlings in a susceptible stage at the time ecological conditions are likely to be favorable for infection is practiced in controlling flag smut of wheat, for example, and may be used for pupation disease of oats (138) and flax rust (148). Many of the virus diseases are dependent on insects for spread. Unless conditions are such that fairly large numbers of the insects may be present and may become infective at a time when the hosts are susceptible to destructive attack, no epiphytotic is likely to develop. Losses from wallaby ear of corn may be greatly reduced or nearly eliminated by planting corn early enough so it will be nearly mature before the vectors become prevalent after midsummer. The beet leafhopper (*Eutettix tenellus* (Baker)), which is the vector of curly top in our western States, is a dry-atmosphere insect by preference. Not only is this insect confined to more or less arid regions, in its natural distribution, but it does not thrive in the beet fields if the beets are large enough to make considerable shade and attendant increased humidity before its winter hosts dry up and force migration to the beet fields. However, the vector has been transported to other areas and may be sufficiently adaptable to become permanently important under different conditions. Spread and development of peanut rosette, transmitted by *Aphis medicaginis* Koch (*A. leguminosae* Theob.), is increased by dry weather, and thick sowing gives partial control. Maize streak was unimportant in South Africa in 1935–1936, when spring rains were light and very late; but infection was nearly 100% the next year when spring rains were early and abundant. The higher humidity increased rather than decreased the transmission of maize streak by its vector.

The pycnidia of the mal secco fungus on lemon are blown about by the wind, and release spores when sufficient moisture is present. In laboratory experiments it was necessary to maintain abundant moisture, either free water or 100% humidity, for 40 hours or more at 15° to 16° C. in order to obtain spore release, germination and infection (109). The banana leaf spot (*Cercospora musae* Zimm.) (73), which has created such havoc in the Caribbean banana-growing areas in recent years, seems to require three or four successive nights of high humidity and moderate temperature (80° F. or lower) in order to infect banana leaves. The foreign *Sclerospora* downy mildews of corn (72, 157, 158, 159) would be unlikely to produce epiphytotics in this country except in areas with warm humid nights, but those are the good corn areas. Some foliage diseases are destructive where the hosts are crowded, with consequent increase in humidity, but are not serious otherwise. Damage from the *Pleospora* which causes drying out of poppy leaves is worse in hot than in cool seasons (112). The nematode causing ufra disease of rice and the one destroying black currant buds are relatively unimportant in the absence of moisture films on the stems (143). If hosts requiring considerable water are grown under irrigation in areas where the air is normally dry, pathogens depending on a humid atmosphere for destructive spread may be found to be unimportant. The relatively dry atmosphere in California's irrigated citrus areas is thought to account for the fact that citrus canker and black spot have never become established there, even temporarily, and that melanose is unimportant. The highest incidence of *Colletotrichum indicum* on cotton is in soils with 30% to 50% of moisture, the optimum for seed germination being 50% (144). Many rusts and other fungi are found in arid regions, but they require the presence of rain, dew or other moisture to start new infections. The potato wart fungus seems to require a short growing season and low soil temperatures to persist; so it should not be feared in the southern States. On the other hand, flag smut of wheat does not appear to be able to exist in cold northern wheat-growing areas.

Some pathogens, such as that of club root of cabbage, do not thrive in alkaline soil, while others, such as that of potato scab, are less destructive in acid soil.

In addition to the more or less direct effects of heat, light, moisture and soil acidity on the pathogen, these factors affect the host and its susceptibility to destructive attack. The presence of moisture in the air may keep stomata open for entry of germ tubes of spores of black stem rust of wheat, banana leaf spot, and numerous others.

A crop barely able to obtain the minimum amount of water for healthy growth may be destroyed by a pathogen that reduces the effective root system by rotting a few roots or root tips, that invades the vascular system and obstructs the flow of sap, or that increases water losses by inducing cracking of tissues, preventing the closing of stomata, or otherwise. Even where soil water is abundant, severe reduction, in one or more of these ways, of the amount used by the host may be destructive. Roots may become soft and easily invaded by water-loving pathogens in the soil if the soil becomes waterlogged. Gumming disease of sugarcane is particularly severe on low, wet or poorly drained soil.

Sudden drying of wet soil may cause it to crack, and thus injure roots and give parasites an entrance point, or may cause the plant to wilt because of insufficient root hairs to take in water, so that pathogens may invade and grow rapidly through the weakened shoots. Shoots injured by cold are favored points of entry for the pathogen causing mal secco of lemon.

Difficulty of control of pathogen. The difficulty of controlling a pathogen will in many cases determine the extent of the damage inflicted by it. If treatments, sanitation, crop rotations, certified seed systems or the use of resistant varieties required to control diseases already here would be sure to control a specific disease not yet established, there would seem to be little to fear from it. However, if, in spite of the control obtained, an additional 5% loss should be caused, the margin of profit might be eliminated and serious financial losses result.

As indicated in the discussion of the effect of ecological conditions, it is sometimes possible to obtain some degree of control by changing planting dates. But if planting dates of a crop are now set to avoid local diseases, pests, adverse weather or soil-moisture conditions, the importance of a new disease might depend on whether or not new and otherwise less desirable planting dates would be required to control it.

When an alternate host is required to enable a destructive disease to persist, control might depend on the possibility of eliminating one host or set of hosts. White pine blister rust is controlled by eliminating currant and gooseberry plants, but the foreign rust (*Cronartium flaccidum*) of hard pines has so many diverse hosts that elimination of all of them would probably be impracticable. Pathogens which, like this rust of hard pines, are able to infect a number of different hosts readily, may be sufficiently adaptable to be able to spread to additional hosts and to handicap efforts to breed or select resistant varieties. Virus diseases seem to be particularly likely to affect diverse hosts.

Virus diseases requiring insect or other vectors or carriers that are not present in this country, in order to spread appreciably, would not be destructive here unless the vectors were introduced also and were able to survive and multiply under our conditions, or unless native insects were able to transmit the viruses before the infected hosts succumbed. The vector *Eutettix tenellus* of the sugar-beet curly top virus in this country has been found to be incapable of transmitting the somewhat similar virus disease that occurs in Argentina. The tristeza "virus(?)" of sour orange stocks can be brought under control in an affected sweet orange tree by top-working it with lemon or sour orange, the leaves of which apparently produce some substance which inhibits any evident action by the virus.

High yields of rubber are expected from *Hevea* trees produced from high-yielding strains susceptible to the destructive leaf blight *Dothidella ulei* P. Henn., but completely top-worked so that the leaves of mature trees will be those of another form which is resistant to this disease (78). Promising results have been obtained with a substance (disodium ethylene bisdithiocarbamate) that is absorbed by plants and makes them lethal to certain pathogens and insects (1, 65, 126). Perhaps further studies on antibiotic substances, fungicides, bacteriophages, ultra-short wave lengths, trace elements, hormones, vitamins and so forth will make elimination or control of now dreaded foreign pathogens easy and inexpensive, and thus reduce their destructiveness to the vanishing point, but no such happy state of affairs can be expected in the immediate future.

POTENTIAL SOURCES OF DESTRUCTIVE FOREIGN DISEASES (19, 128)

Records of diseases found on plant materials coming to our ports (147) show that Italy and Japan have many plant diseases that are not yet established in this country. Italian occupation of African areas for several years and Japanese occupation of numerous islands as well as mainland areas may well have resulted in the introduction and establishment of new diseases in both countries. In Russia the rapid development of agriculture, with the introduction of new plants which are subject to destructive infection by the diseases of native plants, and the employment of a greatly increased number of pathologists to study these plant diseases have led to numerous reports of new diseases occurring there. Acceleration of agricultural development in South America, with increased scientific activity, should be expected to bring to light numerous unreported diseases on that continent.

China with its ancient civilization undoubtedly has accumulated numbers of diseases that, like citrus canker and chestnut blight, would be destructive in this country, even though the cultivated varieties grown in China have been developed through centuries of selection to a high state of resistance. Europe, Africa, Australia and numerous islands continue to report new and destructive plant diseases with disturbing frequency.

EXAMPLES OF DESTRUCTIVE FOREIGN DISEASES

The following examples of destructive foreign diseases of plants show several affecting each general group of hosts, although some of the hosts belong in two or more groups, and some of the pathogens affect hosts in more than one group.

Diseases of plants used for food and feed. Karnal bunt (*Neovossia indica* Mitra) (99) causes up to 20% damage to wheat in the hot lowlands of India. Cool damp weather favors infection. Both spores and sporidia are wind-blown. Wheat flag smut (*Urocystis tritici* Koern.) (44, 71, 72, 101, 118, 149, 168) occurs in Europe, Asia, Africa and Australia, but not in areas having extremely severe winters. Foreign strains in South Africa cause crop losses up to 90% of susceptible varieties. *U. agropyri* (Preuss) Schroet., a smut of numerous grasses in this country and capable of infecting wheat to a limited extent, is said to be the same species as *U. tritici* (44). Whether merely a series of specialized races of *U. agropyri* or races of a distinct species, some of the foreign races of flag smut seem to be capable of being destructive on wheat in this country, should they become established here.

The pupation disease (96, 138, 139), due to a virus, *Fractilinea avenae* McK., carried by the insect *Delphax striatella* Fallen, is thought to have originated in wild grasses, but is widespread on oats and occurs on other cereals and grasses in Russia. Infections of oats run as high as 100%, with almost total loss of grain production.

The ufra disease (*Ditylenchus angustus* (Butl.) Filip.) (21, 43, 52) has caused heavy losses in rice production in India and is reported to occur in Malaya. Rice dwarf (*Marmor oryzae* Holmes) (45, 46, 70, 76, 79) has caused severe food shortages in Japan. The virus is believed to multiply in a vector (*Nephotettix apicalis* Motch. var. *cincticeps* Uhl.) and is carried in or with the eggs for several generations.

In parts of Queensland, Australia, late-planted corn is subject to wallaby ear (116), a virus disease caused by *Galla zeae* McK., carried by *Cicadula bimaculata* Evans and perhaps by other jassids. Infected plants are dwarfed and yields reduced seriously. Maize streak virus (*Fractilinea maidis* (Holmes) McK.) (70, 114, 132, 133, 134, 135) limits production of corn in parts of Africa. Sugarcane and other grasses are less severely affected. A leafhopper, *Cicadulina mbila* (Naude), is the principal vector. Heavy early spring rains are said to increase infections and make losses very serious.

Gumming disease, *Bacterium vasculorum* (Cobb) R. G. Smith (10, 39, 122, 129), prevents growth of susceptible varieties of sugarcane in Australia, South America, East and West Indies, Mauritius and elsewhere, and also infects corn and resistant varieties of sugarcane. Downy mildews (*Sclerospora* spp.) (9, 10, 72, 80, 92, 129, 130, 157, 158, 159) destructive to corn, sugarcane, sorghum and related grasses, occur in Asia, Africa, Australia and Pacific islands. Not one of the five foreign species infecting corn is known to produce resting spores (oospores) on that host. *S. sacchari* T. Miyake in Australia depends on the enormous numbers of conidia produced on corn for destructive spread on sugarcane during the growing season, as relatively fewer conidia are produced on cane. *S. sorghi* (Kulk.) Weston, in India and Africa, produces few conidia and spreads to corn and sorghum largely through distribution of oospores produced in abundance on sorghum. These downy mildews usually require warm humid nights, as are likely to prevail in good corn and sugarcane areas, in order to produce epiphytotics. Under such conditions they may cause losses of up to 100%. The difficulty of germinating oospores artificially has handicapped study of those fungi.

Another sugar plant, the sugar beet, is subject to a virus disease in Argentina (40) that produces symptoms similar to those of curly top, transmitted by *Eutettix tenellus* in the United States. In all tests *E. tenellus* has failed to transmit the Argentine virus. The vector of the virus in Argentina is *Agalliana ensigera* Oman.

The tristeza disease (12, 155), fatal to most varieties of citrus trees grown on sour orange stocks, is destructive in South Africa, Java, Argentina and Brazil. Apparently the leaves of sour orange and lemon produce a substance which inhibits deleterious action by the causative agent, presumably a virus. Black spot (*Phoma citricarpa* McAlp.) (5, 42) of citrus in Australia and the Orient has recently become established in South Africa and Brazil. Where it has been long established losses may be serious, for the fungus causes the fruit to fall and disfigures harvested fruit, often while enroute to market. The mal secco disease (*Deuterophoma tracheiphila* Petri) (42, 50, 109, 110) has killed almost all lemon trees in some areas, and apparently it will eliminate lemons as a commercial crop in the Mediterranean region unless efforts to replace present plantings with resistant forms are successful. Other citrus species are affected less severely. The fungus grows upward through conducting vessels very rapidly; death results in a few months following root infections, but more slowly following twig infections.

A severe and widespread disease of the branches, leaves and fruits of apple in Korea is caused by *Physalospora piricola* Nose (103, 102), which also causes the destructive ring disease of pear fruits.

The brown rots of stone and pome fruits caused by fungi belonging to the genera *Sclerotinia* and *Monilinia* are already well represented here, but additional forms capable of increasing both destruction and the difficulty of control occur elsewhere. Among these additional forms are *M. fructigena* (Pers.) Honey (166), which is serious on both stone and pome fruits in Europe, Japan and Manchuria, and has been reported from South Africa; and *M. laxa* (Aderh. and Ruhl.) Honey f. *mali* (Worm.) Honey (119, 166, 167), which causes blossom wilt and die-back of apple shoots in Europe and Japan. Conspicuous witches'-brooms with stunted leaves are caused by *Taphrina armeniaca* Georgescu and Badea (51) on apricots in Rumania. This disease appears capable of being very destructive.

The black currant eelworm (*Aphelenchoides ribes* (Taylor) Goodey) (43, 52, 97, 127, 143) destroys buds of black currants in England and New Zealand. A film of water on the stems is necessary to enable the nematodes to reach the buds. *A. ribes* has been called a synonym of *A. fragariae,* based on material in leaves and buds of *Grossularia reclinata* from Albion, Calif. If so, it seems to be a different physiological strain.

Court-noué (16) is a destructive virus disease producing such variable symptoms on grape vines that its exact status and distribution in Europe is confused. *Phylloxera vitifoliae* (Fitch) is said to be a vector, but the disease spreads from one root system to another without aid from *Phylloxera.* Plants of American grapes are very susceptible.

Potato wart (*Synchytrium endobioticum* (Schilb.) Perc.) (18, 61, 72) is destructive in areas having cool soil and a short growing season. It occurs in North and South America, Europe, Asia and Africa. Recently two new strains appeared in Germany and were found to be destructive on nearly all resistant varieties developed through years of extensive breeding work. The small amount of potato wart in North America is being carefully guarded and eradicated (61). The potato variety Aucklander Short-Top in New Zealand appears to be healthy, but carries a virus (25) that is virulent on Arran Chief and other varieties, and that causes collapse of above-ground parts, as well as tuber decay. Tobacco, petunia, tomato and some other related plants are susceptible. The virus is said to be a strain of *Marmor dubium* Holmes.

A collection of 59 potato species and varieties taken to England from the Lake Titicaca region in Peru (35) was found to carry a number of viruses and virus complexes, several of them apparently new, that could be serious on our cultivated potatoes. A potato smut, *Polysaccopsis hieronymi* (Schroet.) P. Henn. (170), in South America, forms galls up to five centimeters long and half as thick on above-ground parts. The buba disease (*Thecaphora solani* Barrus), which ruins potato tubers in Venezuela (6, 7) and occurs in Ecuador and Peru, might be destructive here. *Heterodera rostochinensis* Wr. (27, 52), the golden nematode, so destructive to potatoes in Europe, has been found in a few hundred acres on Long Island, N. Y., where it is already reducing yields about 50%. It is hoped that studies under way will show that eradication of this nematode will be practicable.

Pea rust, *Uromyces pisi* (Pers.) D By. (59, 131, 146), occurs in several strains that affect *Lathyrus* spp. and other legumes, including garden peas. The alternate hosts are *Euphorbia* spp. This is an old rust and very troublesome at times in Europe unless control measures, including removal of alternate hosts, are maintained. Pea streak (Pisum virus 3) (24) causes heavy losses of garden peas in New Zealand, infections resulting in discoloration, cessation of growth and ultimate death of plants.

A grass rust, *Uromyces graminis* (Niessl.) Diet. (105), the alternate stage of which is common on fennel (*Foeniculum vulgare*), causes severe necrosis on carrot in Europe. This is a disease that is not usually destructive but might become so if introduced here.

A smut, *Urocystis brassicae* Mundkur (98), produces root galls on and greatly reduces seed formation by *Brassica campestris* var. *sarson,* an important oilseed plant. This smut also attacks radish, turnip, cabbage and related plants in India.

A root rot (*Zopfia rhizophila* Rabenh.) (30, 156) of asparagus is usually unimportant in Europe, but in 1935 it caused losses up to 80% in plantings in a German locality where it had not been noted since it was described from there in 1887.

Elsinoe batatas Viegas and Jenkins (73) sometimes causes a serious stem and foliage scab of sweetpotato in Japan, Guam and Brazil.

A soft rot of squashes in Japan is caused by *Choanephoroidea cucurbitae* Miyake and Ito (91) which also rots flowers within a few hours after they become infected. Later the diseased flowers and fruits mummify.

Diseases of forage plants. A bacterial disease, *Aplanobacter rathayi* E. F. Sm. (38, 39, 122), is at times destructive to cocksfoot or orchard grass (*Dactylis glomerata*) in Europe and sometimes infects rye and one or two other grasses. The few seeds formed on infected plants carry the disease. Diseased seeds produce a poor stand, with distorted or stunted plants on which a gummy exudate glues infected parts together. In May 1945 this disease was found in a United States Department of Agriculture nursery at Corvallis, Ore., on plants of the Akoroa strain of cocksfoot grass grown from New Zealand seed.

The widespread rust of lespedeza in this country is *Uromyces lespedezae-procumbentis* (Schw.) Curt. (68, 69, 141) which does not infect oriental species of lespedeza even though those species are subject to severe attack by several strains of a rust called by the same name in Japan. These oriental strains and other oriental rusts of lespedeza have not been reported from North America.

A red clover disease, *Botrytis anthophila* Bond. (84, 120, 145), in Europe is spread from flower to flower by bees, and reduces seed yields. The mycelium grows under the seed coats of much of the seed that is formed and becomes systemic in plants grown from such seed.

A mosaic (3) due to a new virus which reduces spring growth of *Trifolium subterraneum* by 50% and affects other legumes, including peas, beans, soybeans, sweetclover and *Trifolium* spp., has been spreading rapidly in Australia and may be seed-borne.

Diseases of fiber plants. Leaf spotting and severe stunting of hemp (*Cannabis indica*) in Germany is caused by *Didymella arcuata* Roeder (113).

Flax rust (*Malampsora lini* (Pers.) Lév.) (137, 148, 154) is a serious disease in this country and necessitates the use of resistant varieties. Some of these resistant varieties are highly susceptible to strains of the rust that occur in Argentina. Other foreign strains occur in Germany, Australia and Tasmania.

Vein mosaic (31), a new virus disease, causes stunting of plants and distortion of leaves of species and varieties of cotton in Brazil. A cotton anthracnose, *Colletotrichum indicum* Dastur (33, 144), kills seedlings and ruins bolls in India. Infected seeds may germinate, but death usually occurs while seedlings are young. In infected soil all seedlings may be killed.

Diseases of forest and ornamental trees. A rust, *Cronartium flaccidum* (Alb. and Schwein.) Wint. (4, 15), which causes considerable damage to *Pinus sylvestris* in Europe, is potentially dangerous to our hard pines because its uredial and telial stages may be formed on a wide variety of hosts in several families, so that control by eradication of these alternate hosts would be extremely difficult or impracticable. Among these alternate hosts are peony, nasturtium and verbena. Uredia of the rust were found on *Impatiens balsamea* at the Experimental Farm, Charlottetown, Prince Edward Island, Canada, in 1925, but apparently the rust did not become established.

A number of rusts in this country attack needles or cause witches'-brooms on firs, but there are 50 or 60 additional rusts (67) of firs in various parts of the world that, as a group, have many alternate hosts. The combined effects of some of those species might well be destructive if they became established here.

A white heart rot due to *Polyporus litschaueri* (Lohw.) A. Bond. (13) infects 60% to 90% of the oak *Quercus mongolica* in the Russian Far East, often making the trees useless for timber. Elm, maple and poplar often are affected in various parts of Russia and Austria. A white rot of oak in Japan is caused by *Stereum hiugense* Imazeki (73).

A bacterial canker, *Pseudomonas fraxini* (N. A. Brown) Skor. (14, 121), causes severe dwarfing of a large portion of the ash trees in some areas in Europe.

Pestalotia disseminata Thuem. (58), formerly known from Portugal only, has recently been found doing considerable damage to young eucalyptus trees on the University grounds at Montevideo, Uruguay; a similar and perhaps identical species was found recently in the United States. Seedlings of several species of eucalyptus in Argentina were found to have chlorosis and stunting of the leaves, caused by a graft-transmissible virus (41).

An active leaf parasite, *Stegophora aemula* Syd. (140), on

Ulmus davidiana in China seems to be capable of causing serious injury to elms.

The well known and destructive watermark disease (*Bacterium salicis* Day) (34, 88) of willows in England has not yet been reported in this country.

A needle fall disease, *Exosporium deflectens* Karst. (115), of *Juniperus communis* in Finland and Rumania might be destructive here.

Leaves of *Acer trifidum* in Japan are killed by *Pseudomonas acernea* Ogawa (104) in such numbers as to seriously injure the trees and ruin their appearance. A number of species of maple are susceptible.

Diseases of ornamentals other than trees. The rose-wilt virus (*Marmor flaccumfaciens* Holmes) (55, 54, 70) is especially destructive on roses in Victoria, Australia, occurs in New Zealand, and is similar to a rose virus in Italy. Defoliation and death start at the tips of the plant and continue downward.

Carnation wilt (*Verticillium cinerescens* Wr.) (160, 161) has been destructive in England for a number of years.

A virus or a mixture of viruses (48) causing leaf curl and vein banding of *Ageratum conyzoides* and tobacco occurs in Ceylon. One or more new strains of virus capable of being serious if introduced are suspected of being involved.

A new virus leaf curl disease (82) of petunia occurring in South Africa was found to be transmissible to tobacco, tomato and other hosts.

A rust, *Puccinia distincta* McAlp. (64), is common on *Calendula officinalis, Bellis perennis* and *Senecio cruentus* in Australia and New Zealand. Infected plants are likely to be distorted or defoliated and ruined. In Japan, *Bacterium calendulae* Takimoto (142) is destructive to *C. officinalis,* especially among dense stands during rainy autumn weather, when it causes blackening and distortion of foliage and stems.

Snapdragon downy mildew (*Peronospora antirrhini* Schroet.) (100), first described on wild snapdragon (*Antirrhinum orontium*) in Germany in 1874 and later reported from Denmark and Switzerland, has recently been reported in Ireland (1936) and England (1937), where it has caused the destruction of large plantings of *A. majus,* the species grown in garden and greenhouse. The disease was found in New South Wales, Australia, in 1941.

Wild windflowers, *Anemone* spp., in Germany are badly distorted and ruined by a virus, *Galla anemones* Holmes (70, 77). Diseased plant parts in the soil carry the disease. How destructive the virus might be on related plants in cultivation is not known.

The destructive dahlia smut (*Entyloma dahliae* Syd.) (53, 83) is widely distributed over the world and has recently been found on a few plants in Oregon.

A leaf spot, *Cercosporella inconspicua* (Wint.) Hoehn. (93, 169), is a limiting factor in the growing of lilies in the Ukraine. The fungus is reported from elsewhere in Europe and from Japan.

In Africa wild *Gladiolus* spp. are severely injured by rusts (*Uromyces* spp.). A new rust, *Puccinia Mccleanii* Doidge (37), was recently described on *G. ludwigii* in South Africa.

Diseases of special or multiple-purpose plants. A rust, *Uromycladium tepperianum* (Sacc.) McAlp. (65, 128), sometimes destroys commercial plantings of *Acacia pycnatha* in Australia. The host is grown for tannin, perfume and gum, and is used as a sand binder. We do not know what the rust would do on acacias and related plants grown in North America.

Peanut rosette (*Marmor arachidis* Holmes) (124, 136) inhibits seed formation and is often destructive, crop losses being as high as 90% in some areas. It is reported from Java as well as from various parts of Africa. It is transmitted by *Aphis medicaginis* and perhaps other insects.

Soybeans grown in the Russian Far East are subject to destructive attacks by *Ascochyta sojaecola* Abramoff (2). A stem break caused by a *Fusarium* sp. (2) killed 9% to 22% of the young plants in the same area. In another part of Russia the *Fusarium* killed a large portion of the seedlings which were planted with seed from the Far East.

Lavender plantings in England are devastated by shab disease (*Phoma lavandulae* Gab.) (20, 57, 87) unless careful sanitation is practiced. Goosefoot (*Chenopodium album*), common as a weed in lavender plantings, is a host of the fungus.

A destructive root rot and wilt of pyrethrum (*Chrysanthemum cinerariaefolium*) on the island of Cherso, Italy, is caused by *Vialina radicicola* Curzi (32).

The oil and opium poppy and related forms are subject to a destructive seed-borne bacterial blight, *Bacillus papaveri* Christoff

(29, 36), in Bulgaria. *Pleospora calvescens* (Fr.) Tul. (28, 36, 112) causes drying out of poppy leaves and is especially destructive in Bulgaria. The fungus occurs elsewhere in Europe and in Japan.

A destructive leaf spot of tung trees in China is caused by *Mycosphaerella aleuritidis* (Miyake) Ou (108). It has been reported from Brazil, and a form of *Cercospora* found on tung trees in Florida may be the imperfect stage of this fungus.

The most severe damage to cork oak (*Quercus suber*) in Morocco is caused by *Hypoxylon sertatum* Dur. and Mont. (81) which starts on the outermost twigs and grows down to the trunk, after which the tree soon dies. The fungus has been reported on cork oak and walnut in Algeria and once on eucalyptus in Morocco.

Stevenson's manual (128), issued in 1926, lists several thousand diseases that are not established on the North American mainland, and numerous others have since been reported. Recently the present writer supplied plant-quarantine inspectors with revised and amplified notes regarding foreign diseases of some of our more important host plants. Over half of the approximately 1,700 pathogens and possible pathogens listed for the few hosts covered were not included in the manual. Doubtless the increase would have been greater but for the fact that in recent years world conditions have interfered with plant-disease studies and disrupted publication and receipt of such information. Some of the diseases mentioned in this paper are new, and only preliminary reports are available. None has been studied thoroughly enough to make it possible to predict with certainty its action on American-grown host varieties, under the varying conditions in North America, if or when it becomes established long enough to attain stabilized destructiveness.

GENERAL DISCUSSION AND CONCLUSIONS

Foreign plant diseases may enter with plants or products imported for food, feed or manufacturing purposes, or for propagation; with packing materials such as straw and rice hulls; or with bouquets or decorative plants. Pathogens may be present in or on these plant materials, and it may be impossible to detect them by a practicable method of inspection. Viruses are often carried by hosts in which no symptoms are produced at any time, as in some potato and raspberry diseases. Insect stowaways carrying viruses may escape notice in bulk shipments which receive sample inspection only, or in merchandise or products which are not examined by quarantine inspectors. Oospores of downy mildews, and spores of rusts, smuts and other fungi, either individually or in leaf or stem fragments, are likely to be mixed with grain or other seeds from nearby plants as well as from infected hosts. Infected sugarcane is often planted. Potato tubers infected with virus or tuber rots, or contaminated by adhering fungus spores, bacteria or nematodes are used for planting purposes. It is necessary to know something of the methods of reproduction of a pathogen and the possibility of its occurrence in viable condition in, on, or with the parts of the host that are imported in order to appraise the likelihood of its entry. The evidence is clear that many destructive plant pathogens have been and others may be spread with plants and plant parts or products as imported. The greater speed of transportation and improved conditions of storage in transit greatly increase the likelihood of undetected importation and ultimate establishment of foreign pathogens[3].

It is obvious that destructive foreign diseases abound; that they are of many types, not only as to their taxonomic positions, but as to the hosts and host parts affected, their effects on the hosts, their modes of spread and survival, preferred ecological conditions, methods of control, and possible methods of entry; and hence that preventing their entry for as long as possible and mitigating the effects of their entry are difficult and complicated problems. Every useful plant is entitled to the maximum protection that is profitable; all of us receive direct or indirect benefit from such plants and are entitled to have our interests protected.

Needed protection may be attained by maintaining a planned program to include the following:

(*a*) Thorough study of foreign plant diseases so that we may know what they are; how they grow, survive and spread; how they affect all parts of their hosts; what varieties they attack; how they can be detected on material destined for this country; and how they can be eliminated therefrom.

(*b*) Use of quarantines drawn on the basis of biologic and economic facts, use of the most highly developed techniques for inspection and treatment of incoming plant material, including, when desirable, growth of propagation material under observation until found to be disease-free.

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[3] *See* McCubbin, W. A. Preventing plant disease introduction. Bot. Rev. 12: 101–139. 1946.

(*c*) Use of plant disease surveys to find diseases before they can become widely or perhaps permanently established, and thus to make possible their early eradication.

(*d*) Development of eradication methods for different types of diseases, crops and conditions, so that eradication may be effected promptly and economically, if practicable.

(*e*) Development of control measures, including good resistant varieties or breeding stocks, in anticipation of possible establishment of destructive foreign plant diseases that are likely to be introduced.

In general, we should obtain the data needed and then take the steps necessary to protect ourselves efficiently and effectively from needless economic and esthetic losses of plants and plant products through the ravages that otherwise will follow the inevitable arrival of destructive plant diseases not yet established in North America.

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Only one citation is given for each disease in the list if the one paper covers most of the information used regarding the disease. Several citations were needed in some cases. Additional citations will be found in many of the papers listed. Although not complete, the literature on foreign plant diseases is well covered and indexed in the Review of Applied Mycology.

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MARINE ALGAL ECOLOGY

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INTRODUCTION

The ecology of marine algae has now reached a stage, marked by the accumulation of a considerable volume of literature, where some attempt at integration is desirable. Plant and animal ecologists have generally pursued somewhat different paths, but it is impossible for the marine algal ecologist not to take cognizance of the work of his zoological co-workers. It is of course possible to carry out marine algal studies without regard to the associated animals, but such work can be regarded only as incomplete. The necessity to consider both plants and animals (46) has rendered the subject fraught with more difficulties than it would otherwise possess, but it is impossible to avoid them. It is hoped that some of these difficulties will become apparent in the following pages.

It is convenient to commence with a brief summary of the more important descriptive or synecological studies, since these, from a chronological point, were first in the sequence; only later did autecological studies make their appearance. Generally speaking, marine algal ecology is still in the synecological stage, and autecological studies are only just beginning to appear.

It has been evident for some time that algal communities on sea shores can be grouped into certain broad regions, each of which is most suitably regarded as a whole. Thus the Atlantic, Arctic and North Sea coasts of Europe are akin in their vegetation with the shores of Iceland and eastern North America. Similarly the coasts of western North America, Japan and Siberia form another entity. Both these major areas are characterised by predominance of large brown algae belonging to the Laminariales or Fucales. In tropical and subtropical warm waters brown algae cease to be so important and the Rhodophyceae are more conspicuous. In the warm waters one may distinguish four more important regions—Caribbean, Mediterranean, Indo-Malay, Central Pacific—whilst there may be other smaller units. In the colder waters of the southern hemisphere we again encounter an area where the larger brown algae predominate. At least two major separate regions can be distinguished there, South America and New Zealand. Although the larger brown algae predominate in the floras of northern and southern cold waters, nevertheless their aspect is very different in the two regions because the species are commonly totally distinct. One may also observe that in colder regions the littoral[1] vegetation is extremely well developed, whereas in warmer waters it is the sublittoral that is more luxuriant. Conditions in the littoral militate against survival of delicate algae during periods of low tides in the tropics and subtropics, and hence the vegetation tends to be poorer in number of individuals and of species. There is also the seasonal fluctuation in the algal vegetation. Thus in the north temperate region the algal flora is at its height in July and August, but in the Mediterranean it is at its best during winter and spring, though the sublittoral is maximal in summer when the littoral is strongly reduced.

In the north and south temperate regions the flora has frequently been studied in relation to the nature of the coastline (49). Thus one can consider the vegetation of rocky shores (exposed or protected from wave action), estuaries, sandy shores, muddy shores and salt marshes. A similar classification can also be employed for tropical vegetation, except that mangrove swamps occur in many of the areas that otherwise would be salt marsh. Because of their peculiar nature and also because of differences in their environmental factors it is desirable to consider independently the rock pools of rocky shores and the salt pans of salt marshes. Most of what immediately follows applies principally to the algal ecology of rocky shores (74).

ATLANTIC EUROPE—EASTERN NORTH AMERICA

A considerable volume of work has been published dealing with the algal vegetation of this region (1, 5, 8, 10, 21, 22, 27, 34, 42, 44, 46, 49, 53, 56, 70, 73, 84, 85, 88, 90, 91, 97, 101, 102, 109, 115, 116–119, 123, 125, 126, 128, 139, 145, 149, 151, 152, 156, 177, 185, 189, 191, 192, 194, 196–199, 206, 208, 224, 225, 226, 228, 262, 263). The number of communities recognised has depended partly upon personal predilections and partly upon the amount of time expended upon a survey: the longer the time the greater the number of communities usually described.

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[1] For definition of the Littoral, cf. p. 654.

At the outset certain important conclusions can be drawn. Thus, it is very evident that the zonation to be observed in chalk areas (1) differs markedly from that of other regions, whilst the vegetation of the Baltic (145) also exhibits peculiarities based upon its tideless nature and gradients in salinity. Another feature is the part played by ice formation in the Arctic and Antarctic, and also the Baltic, in preventing some species from growing on the littoral (232).

Many of the communities described from the different shores are not of equal ecological status, and this renders any attempt at comparison extremely difficult. If future workers accept normal ecological practice as the basis of their nomenclature it will greatly facilitate comparisons of floras from different areas. One attempt at equating the different algal communities on British shores has been made (38), and it seems clear that there is a more or less basic zonation which can be found in the majority of localities. The very top zone in the spray area is generally dominated by *Hildenbrandtia* and *Verrucaria;* below this at about E.H.W.M.S.T.[2] there is often an upper *Enteromorpha* belt, usually with other associated Chlorophyceae, or alternatively a *Porphyra* belt with *Bangia* and *Urospora.* Then comes a zone covered with plants of *Pelvetia canaliculata* followed further down by *Fucus spiralis* or its variety *platycarpus.* The next two successive plant belts vary in their position according to local factors so that the balance between the two species *Fucus vesiculosus* and *Ascophyllum nodosum* must be very delicate, and indeed in many areas they commonly occur as a mixed community. The next lowest fucoid zone, near L.W.S.T., is dominated by *Fucus serratus,* though in certain exposed areas one may find all these lower belts replaced by a single zone of *Himanthalia lorea* (73) or a *Gigartina-Chondrus* community. Farther north, *e.g.,* the Shetlands, slight changes occur in the basic zonation (21); *e.g., Fucus serratus* is absent, but minor changes of this category occur elsewhere (109). Around M.L.W.S.T. one frequently finds also a lower *Porphyra* community and a *Laurencia* community. In the sublittoral *Laminaria digitata* commonly forms the uppermost belt and it is usually succeeded, at two to three fathoms, by *L. cloustoni,* the lower limits of which are extremely variable. It is said to be determined principally by the degree of light penetration (77), but there is little doubt that complex interactions are also involved.

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[2] Extreme High Water Mark Spring Tides.

The vertical extent of the zones on any shore is directly correlated with the angle of slope of the beach and the extent of the tidal rise. This latter effect is very pronounced and can be demonstrated by a comparison of zonations from different areas (34, 264). The zonation in any area may vary during the seasons (123), and this is also a fact which must be taken into consideration though it is one that has not received as much attention as it deserves.[3]

NORTH PACIFIC

This area has been little studied ecologically, apart from an investigation into the distribution of the great kelp beds (31) and an analysis of the geographical elements (179, 217). There is no doubt that in it a very fruitful and profitable field is still awaiting investigation. Future work will probably have to be based to some extent upon that of zoologists (2, 223), though it is probable that a new approach will be necessary because too much insistence has been placed upon the rôle of animals. So far four biomes have been recognised, all dominated by animals, and even the great kelp communities are treated only as associations or fasciations of the animal formations. It seems clear that a combined approach by both zoologist and botanist should do much to determine the effective status of the different communities.

SOUTH PACIFIC

New Zealand. The nature and composition of the algal communities in these cold waters differ from those of the northern hemisphere. The area is very rich, but so far only a few ecological studies have appeared. In one of these (180) eight formations were recognised, each comprising a number of subformations and associations characterised principally by either algae or animals but not by both. Later work (50) has rectified this outlook and shown that very often the animals and plants should be regarded as co-dominant. A spray zone can be present, but no data are provided of its extent. It is perhaps significant that the two principal marine ecological studies from this area have been based upon the biotic concept of natural communities (biomes). A definite zonation of both plants and animals has been described, the principal communities being briefly as follows: above H. W. M. in the spray zone there is a *Verrucaria* and Cyanophyceae belt, whilst the top of the littoral is occupied by *Chthalamus;* below this the sequence is first an *Apophloea-Elminius* association, then a *Novastoa*-encrusting coralline association, and finally a belt of *Xiphophora* and *Carpophyllum elongatum;* in the sublittoral there is a Rhodophyceae association, a *Lessonia* association and in places a *Macrocystis* association; these may all be displaced downwards to some extent in areas where there are both shade and tumultuous waters.

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[3] A seasonal succession is as follows (151): Oct.: barren; Nov. to Jan.: *Ilea-Scytosiphon-Dumontia-Polysiphonia;* Feb. to Ap.: *Monostroma-Spongomorpha-Bangia;* May, June: *Laminaria-Chorda-Polysiphonia;* June: *Chordaria;* Aug. to Sept.: clearance of all annuals.

South America and the Antarctic. Our information about the algal ecology of this region is extremely meagre and is largely based upon the work of a single contributor (230–232), though there are, of course, a number of flora lists. There is, therefore, a vast potential field that requires intensive study, and there is now a sound basis upon which further work in subantarctic and antarctic waters can be built. Some of the formations described will need modification, and zoologists will have to contribute their quota in order to complete the picture. A large number of associations within the formations have been recognised, and, as may be expected, there is a marked difference between protected and exposed coasts. One of the more outstanding features is the very large number of communities described from the sublittoral, a condition reminiscent of the tropics. The littoral vegetation is relatively poor in communities, due to ice removing many of the plants and so preventing a number of the perennials from becoming established.

Throughout the South Pacific there is a marked absence of dominant fucoids in the principal zonations, though *Hormosira banksii* may play a subsidiary rôle in New Zealand, whilst on exposed coasts *Durvillea antarctica* sometimes forms a community near low water mark. The subantarctic sublittoral is characterised by associations of the giant kelps (*Lessonia* spp., *Macrocystis pyrifera, Durvillea harveyi*) but these are unable to penetrate into the even colder waters of the antarctic where *Desmarestia, Leptosarca* and species of Rhodophyceae form associations that have yet to be studied in detail.

SOUTH ATLANTIC

We owe our knowledge of this region mainly to the series of excellent works (30, 61–63, 107, 146, 169, 236, 237, 240, 241) on the South African inter-tidal zone. These have been summarized (236, 237) in order to emphasise the general picture. It is clear that this type of investigation, in which a large area is selected and studied over a long period of years, will yield important results and information of the greatest value, and co-operative work such as this should become the rule rather than the exception. The vegetation of the shores varies in passing from the warm waters of the east coast round the south coast to the cold waters of the west coast, and the transitions from the two extreme zonations on west and east have been carefully traced. This change in zonation is dependent very largely upon the temperature relations of the various species. Apart from the principal changes in the zonation there are also local changes associated with the exposure or protection of the coast (237).

On all three coasts there is a well marked upper *Littorina* belt, though certain associated species become more abundant on the warmer east coast. Below is an extensive balanoid zone which is less thickly populated on the west coast. The lower part of this zone is occupied by *Patella argenvillei* on the west coast, but on the south coast it is represented by a distinct *Patella cochlear* belt. On the east coast both species of *Patella* gradually disappear and are replaced by a carpet of red algae. The lowest zone, or sub-littoral fringe as it is called, is mainly composed of Rhodophyceae on the east coast, whereas on the south coast it is dominated by the crinoid *Pyura* and in a few places by *Macrocystis pyrifera.*

It will be seen from this brief account that there is a relatively simple basic zonation which, it is believed, can be applied to other areas (236) outside South Africa, *e.g.,* Scotland, where it is possible to demonstrate an upper *Littorina* zone and a lower balanoid zone, together with a sub-littoral fringe. Terminologically the name sub-littoral fringe is hardly satisfactory, and since it varies so widely in composition from place to place future work is likely to necessitate its division into formations and associations. Additional studies will also be necessary before it can finally be accepted that the *Littorina* and balanoid zones have an extensive distribution. In view of the fundamental differences between the algal vegetation of the northern and southern hemispheres it is doubtful that the relative insignificance attached (236) to the fucoids in northern waters is justified.

CARIBBEAN REGION

Ecologically this area has been but little studied, although lists of the marine algae to be found in parts of it are in existence (20, 23–25), whilst there is a fairly extensive study of the algal associations and facies of the Azores (207), which can be included in this region. There is reason to believe that lime-depositing Cyanophyceae may play some part in the building up of the shore line (18), though not perhaps to the extent they do elsewhere. In spite of the extremely small tidal range of the area one can find perfect examples of algal zonation, albeit on a reduced scale (41), and there is no doubt that a big field awaits investigators both here and in the following region.

INDO-PACIFIC REGION

(Including Oceania and tropical Eastern Australia)

A little information is available about algal zonation on a Ceylonese reef (244), on the Great Barrier Reef (239) and also on Hawaii (153) and Tahiti (216). This region and the previous one suffer from the fact that most of the work has been carried out by visiting scientists, and no real advance in their ecology is likely to be made until workers who are resident in the tropics inaugurate some investigations.

THE MEDITERRANEAN

This area is fortunate in possessing a well equipped marine station at Naples and also in having suitably located Universities. In addition, the shores are readily accessible to workers from other European Universities during summer vacations. As a result, a number of ecological investigations of very high value (12, 60, 64–67, 147, 181, 187, 204, 205, 209, 218–220) have been forthcoming. In some of these the biotic concept has been adopted as a basis for description of the communities, but the majority of the investigators have been concerned either with plants or with animals.

A very large number of associations have been recognised, though many of them would not enjoy this status if proper ecological terminology were applied. In the supralittoral seven communities have been recognised at Albères including *Verrucaria* and *Hildenbrandtia* (66). The number and nature of the littoral communities depends on whether the coast is exposed or sheltered, but in either event there is a lack of dominant fucoids, and their place is occupied by *Bangia, Porphyra, Rissoella, Tenarea, Nemoderma, Corallina, Ralfsia, Cladophora, etc.* As might be expected in warm waters, the number of communities to be recognised in the sublittoral is large, no less than 12 in one case (66). The most characteristic feature of the sublittoral is the presence of a number of communities, each dominated by a species of *Cystoseira.* Areas with muddy or sandy bottoms are dominated by marine phanerogams, a group which tends to play a much larger part in the ecology of warmer waters. Different profiles (66) have been used to illustrate the variations of zonation that can be found under varying conditions of slope, shade and aspect. There is no doubt that the Mediterranean represents the most fully studied warm water region, and some effort should now be made to bring our knowledge in tropical regions up to the same degree of excellence.

One of the problems up to the present has been the difficulty inherent in preparing an accurate survey of sublittoral vegetation. The exigencies of two successive wars, because of the commercial value of oarweeds and giant kelps, have done something to stimulate research. Between 1912 and 1914 a detailed survey was undertaken of the kelp beds of the Pacific Coast of North America (31), the work mainly being carried out by means of surface craft. Similar methods have been employed during the present war to survey the *Macrocystis* beds around New Zealand (188), but very different techniques have had to be introduced in order to survey the submerged *Laminaria* beds of Great Britain (40), and the use of the echo-sounder and aerial photography may mark a new era in algal ecology. In some cases it is even possible that differences in rockweed vegetation can be seen by air photography, but further research will be necessary in order to show whether any use can be made of these two techniques in warm waters. Using these methods large areas can be surveyed and mapped in a relatively short time, and as a result it has been found that the lower limit of *Laminaria cloustoni* is not uniform around Scotland. This fact, when considered in the light of our knowledge of the physiology of the alga[4] and the different types of environment in which it occurs, can illuminate certain ecological problems; and if it does not succeed in providing a complete solution, it nevertheless certainly

[4] There are three physiological races of this species (160).

indicates the lines along which a solution should be sought, not only for this species but also for others.

ROCK POOLS, CAVES, SALT MARSHES AND PLANKTON

The vegetation of rock pools has received little attention, and there is plenty of scope for additional work. The flora of pools in various areas has been described (13, 49, 52, 54, 66, 88, 99, 109, 112, 120, 134, 191, 229, *etc.*), and certain differences noted as compared with the remainder of the beach vegetation. Hence much of the flora is transient (11), and there is often a microzonation around the walls of the pool. Three different types of rock pool have been recognised (66, 88), depending on their vertical elevation on the shore. These three categories are: *a*) sublittoral pools characterised by protection from surf and water currents, *b*) littoral pools, *c*) supralittoral pools of the spray and supralittoral zones. This last group often has a layer of fresh or brackish water floating on top of the denser salt water, and they may also contain peculiar forms of well known species (*e.g., Enteromorpha* at Nahant, Mass., and *Fucus serratus* in the Orkneys found by the writer). The conditions in these three types of pool are very different, but more data are required in order to place these differences on a quantitative basis. Some attempt in this direction has indeed been made for pH and temperature variations (120) where a gradation was recorded from pools dominated by Chlorophyceae and showing considerable fluctuations to pools dominated by Rhodophyceae with very small fluctuations.

Various authors (15, 49, 52, 120, 134–137, 229) have made suggestions concerning the nature of the factors that control the vegetation in these pools, and the following seem to secure general agreement: *a*) height of pool on shore, *b*) depth of water in pool, *c*) size of pool, *d*) drainage action of surrounding algae, *e*) isolation, *f*) temperature changes, though this may affect certain species only (15), *g*) salinity changes, *h*) biotic interactions, *i*) light penetration. pH is not regarded as a limiting factor (15, 119) because the majority of algae will tolerate a very wide range, but slope may affect the density of the cover (229). Although the above list may seem imposing, much of the work has been empirical, and there is still scope for further intensive study.

The elements composing the flora of rock pools have been seriously studied in only one locality (13) where it was found that there were four components: *a*) species characteristic of the sublittoral, *b*) species from the ebb line that reach their upper limit on the beach in the pools, *c*) littoral species, *d*) species wholly confined to pools. One interesting feature is the discovery that algae from tide pools have a greater range of osmotic tolerance than species from the sublittoral, but this is perhaps to be expected, since salinity changes in rock pools may be very considerable.

The ecology of cave vegetation has received some attention (1, 49, 133, 134, 141). but additional data will be of considerable value. Shade species are common on the walls of caves, and certain forms are capable of penetrating a considerable distance into a cave. The ultimate limit of penetration is set by the amount of light within the cave. Both micro- and macro-climates are considerably different in a cave as compared with the rocks outside, and this naturally exerts an effect on the vegetation. Some information is available about these factors (92, 133, 134), and it seems that light and humidity are probably the two most important.

On salt marshes the pans correspond to the rock pools of rocky shores and to the sand pools (49) of sandy shores. Much of the pan vegetation is transient and adventive, but there is no doubt that a definite pan flora can be recognised (34, 37–39). Our information concerning this flora is at present extremely meagre because it is a habitat that has been neglected by ecologists, although it is very suitably circumscribed for ecological study. Similarly our knowledge of the environmental conditions in such places is almost lamentable, although it is known that there are great changes in salinity and temperature and that these may occur at different depths in the pans (174). During the summer pans on the upper marshes may dry out (37), and then the concentration of salt rises until it crystallises out on the soil. The oxygen and pH factors are, as might be expected, controlled by the amount of animal and plant life present (174).

Some of the species that occur in salt pans are somewhat unexpected because they are more typical of rocky shores. In the pans they grow attached to the mud banks or to the exposed roots of phanerogams (34). Examples of such species are *Colpomenia sinuosa, Striaria attenuata, Polysiphonia fibrata, P. elongata* and *Myriotrichia filiformis.*

On the salt marshes proper fewer ecological studies of the algae have been attempted than on rocky shores (38). Well marked communities can be and have been distinguished (32, 34, 36, 37, 49, 110, 177, 191), and suggestions have already been made for coordination of nomenclature on a proper ecological basis (34). So far only the algae of salt marshes on the Atlantic or North Sea coasts of the northern hemisphere have been investigated in any detail, and it is noticeable that there is a general similarity in their floras. One of the most interesting features of the salt marshes is the presence of free-living or embedded marsh fucoids (7, 32, 34–37, 49, 85, 131, 145, 150, 233, 234). These have originated from normal attached forms, but in some cases they have become exceedingly modified. All the common littoral fucoids, including *Fucus serratus* (173) and *F. ceranoides,* appear to have given rise to these free-living forms, though their origin has by no means been satisfactorily elucidated and the distribution of the different types on salt marshes has yet to be worked out thoroughly. One of the more interesting recent discoveries is that of the existence of a comparable free-living form of *Macrocystis* (265), but at present there is very little information about its environment.

The distribution of the various algal communities in time and space (32, 37) brings out interesting features, illustrating not only the seral nature of some of the communities but also their relationship to physiographic features of the salt marsh or to the phanerogams. Cyanophyceae are often pioneers on the lower mud flats, and they are also more common on the upper marshes where their gelatinous nature protects them during long periods of desiccation (37). A comparison of the vertical distribution of species common to salt marsh and rocky coast shows that very often there may be divergences which provide a clue to the factors controlling their distribution (37).

The field of salt marsh algal ecology has barely been broached as yet, and it is to be hoped that future workers will not neglect it as in the past. Even more seriously neglected is the algal ecology of the tropical counterpart of salt marshes, mangrove swamps. Not only do new species probably remain to be discovered from such habitats, but at present we know nothing about the vegetation with any degree of completeness (68). At least one very peculiar plant has been recorded (251) from the Indo-Malayan mangrove forests.

It is barely possible to embark upon the ever-growing body of literature concerning the ecology of plankton. One of the more striking features is the dominance of Cyanophyceae in warmer waters (77), whilst the relationships between planktonic algae and food fish are slowly being elucidated (202). Plankton, however, forms a topic which demands separate treatment; hence no further details will be given here.

ENVIRONMENTAL FACTORS

The geographical distribution of the majority of algal species is controlled by temperature (108, 211–214), often in relation to the production of fruiting organs (*e.g.*, Laminariaceae), many species tolerating only a narrow range. Thus in the Adriatic the distribution of *Fucus virsoides* is controlled in this manner, the plants gradually becoming smaller towards the south and finally disappearing as the mean average sea temperature rises (205). One result of this type of relationship is that the same species occurring in different areas may flourish at quite different periods, *e.g.*, some Mediterranean winter species occur as summer species in the English Channel (66). However, this correlation between resistance to warmth and distribution does not always exist (16), and it is therefore evident that each species must be investigated individually. Apart from this principal temperature relation the distribution of algal species may be controlled locally by the operation of other factors (200); thus in the Baltic lack of tidal movement and salinity gradients are of considerable importance (145). In such land-locked areas it is customary to find a reduction not only in the total number of species but also in the size of the actual plants. In the Arctic and Antarctic the shore vegetation is restricted to species that can survive freezing and ice action every winter (232). It is, however, somewhat surprising that there are very few annual species in these two regions. The above are probably the main factors controlling geographic distribution, though others may function locally (129), but the presence or absence of a species in any given area is largely dependent upon the existence of a suitable substrate; *e.g.*, kelps will not be found in abundance where there is a sandy bottom, whilst the vegetation of chalk is very different to that of a granitic or felspar coast (1, 34).

The vertical zonation of plants and animals that can be observed on most of the rocky shores of the world has stimulated many investigations in order to determine the factors responsible for the phenomenon. In a recent symposium (39, 46, 55, 238) certain aspects were examined and it was generally concluded that although we are approaching some understanding of the primary causes, the picture as yet is far from complete.

It would seem that there are three groups of factors operating on a rocky shore: firstly there are those which actually determine the upper and lower limits and which can be termed the causal factors; secondly there are those which determine whether a given zone shall be present or absent in a locality, *e.g.*, heavy wave action: these may be called presence or absence factors; thirdly there are the *modifying* factors which are responsible for modifying the breadth or vertical position of a zone, *e.g.*, spray or seasonal temperature changes.

Locally along European shores severe wave action may act as a presence or absence factor in eliminating *Ascophyllum nodosum* and *Fucus vesiculosus*, whilst a sand covering on the rocks results in the introduction of a number of other species, *e.g.*, *Sphacelaria radicans* (49); a small rivulet of fresh water may break up the fucoid zonation completely and replace it by a vertical zone of *Enteromorpha* and *Cladophora*, though if the stream is large enough *Fucus ceranoides* may replace the other fucoids. Generally speaking, normal variations in concentrations of nutrient salts are not important, but an increase of nitrogen in the vicinity of sewage outflows results in the development of an abundant chlorophycean flora (*Ulva* and *Enteromorpha* (48)).

It is often easy to note the operation of local factors, and though the majority of investigators (6, 12, 49, 56, 80, 85, 91, 168, 264) are agreed that in the littoral zone the principal causal factor is exposure, at present there is no agreement as to how exposure operates or should be measured. On European coasts it is at the upper limits, where conditions are extreme, that one can find species (*Bangia fusco-purpurea, Urospora penicillus*) that can tolerate very high temperatures and severe drying. Exposure of the seaweed thallus to the air permits it to undergo considerable water loss (91, 104, 105), and in temperate regions this is probably an important limiting factor. In the tropics the heating of the thallus to a temperature that might kill the protoplasm, certainly of more delicate species, may be more important than the water loss, though there are some red algae (*Rhodomela crassicaulis*) which do not suffer damage from this (77). It is not yet established whether water loss acts directly, because as the water is lost, the salinity of the surrounding medium and also the osmotic pressure of the cell sap rises, and this may be the ultimate factor (13, 14) at higher levels on the shore, although the osmotic pressure in certain species is dependent upon the wave length of the light (16). The algae so far studied fall into one of three groups so far as resistance to changes of osmotic pressure are concerned, and their grouping is directly related to their vertical position in relation to mean sea level. There is no doubt that each species will have to be investigated individually because it is extremely unlikely that a common causal factor controls the vertical distribution of all the littoral species.

So far as measurement is concerned, exposure can be estimated in terms of hours of submergence or emergence or the ratio of the one to the other. This type of relationship has been worked out for at least three localities (45, 91, 110). At this point, however, one must distinguish between inter-tidal and non-tidal (39) exposure, and it is probable that the maximum periods of the latter (which may extend into numbers of days, especially in the summer) are of far more significance than the former. Unfortunately this factor has been studied only on salt marshes, and no doubt when it comes to be investigated on a rocky shore it will provide some very significant results. Another aspect of desiccation that will certainly prove to be more important than any so far studied is its relationship to the establishment of sporelings. We know very little about the response of sporelings to varying degrees of exposure, yet it must be at this stage or at germination, rather than at the adult stage, that control of zonation is effected. One or two workers (12, 28) have indeed drawn attention to this aspect, but no adequate data are as yet available, possibly due to the very real difficulty of identifying sporelings accurately at an early stage. We may say that exposure in a general sense can be regarded as one of the prime causal factors, especially in determining the upper limit of species.

The tidal phenomena associated with a littoral beach vary considerably with height on the beach, and attempts (45, 52) have been made to ascertain whether there are not certain critical levels which mark the upper or lower boundaries of the majority of species on the shore. There is no doubt that this method of approach is likely to lead to important results, but until data from more areas are forthcoming it is difficult to assess the probable outcome. The inherent difficulties can be appreciated when we note that the two workers who have approached the problem from this angle recorded only one critical level that was common to both areas. The analysis can perhaps be carried somewhat further by equating weed zones from different localities to a common basis, *e.g.*, the tide (39, 264). This operation shows that the algal zones do not always bear the same relation to the tidal levels in the different localities, but it also suggests that for the fucoids there may indeed be three critical levels. If the problem is attacked along similar lines in other areas there is little doubt that some substantial progress will be made.

Of the other factors that operate throughout the littoral the mechanical effect of the waves is likely to be a presence or absence factor, affecting both abundance and composition of the flora. Certain species require the vigorous aeration associated with surf, *e.g.*, *Postelsia* (216, 235) and *Valonia* (242), whereas others do not. On the other hand, the velocity of the tidal current during ebb and flow may be a causal factor, though further investigation is desirable. There is usually little or no current around high or low water, and the maximum current is usually between the third and fourth hours of rise or ebb. It is a fact that in many areas *Ascophyllum nodosum* and *Fucus vesiculosus* occupy this zone that is associated with rapid movement (39, 52, 59), though whether the lower limit of *F. spiralis* and the upper limit of *F. serratus* are also related to these movements has yet to be established.

It has already been pointed out that the geological nature of the rock may behave as a presence or absence factor, but the angle of slope may act similarly or as a modifying factor in enabling, through shade, a zone to be elevated slightly. Slope is also important in connection with the rate of drainage and hence of desiccation, but this has scarcely been worked out in any quantitative manner.

Spray acts as a modifying factor in that it may elevate some of the upper zones (45), whilst the actual extent of the tidal rise also operates similarly in that it controls the vertical extent of the zones on the shore. Temperature acts in a similar way because seasonal migrations of species that form distinct belts have been recorded (123, 165). In temperate regions exposure to unfavourable low temperatures may also produce a temporary modification in the zonation, but it is dependent upon a low tide coinciding with a severe frost (39, 52, 127).

Several workers (12, 57, 82, 222, 230) have suggested that light may be an important causal factor, and in extremely silty areas this may be so, whilst for *Fucus evanescens* (82) there is evidence that young plants soon sicken and die if the light intensity is reduced. Temperature, light and salt concentration may all influence germination of *Fucus* oospores (122) and this type of investigation wants extending so as to include other genera. Further work is needed on this factor in spite of the tendency to discount it during the last decade or so. We now know that many algae behave either as sun or shade forms (137, 162, 163, 221), and this fundamental physiological distinction may underlie some of the zonations. Some of the fucoids bear bladders, *e.g.*, *Fucus vesiculosus* and *Ascophyllum nodosum,* and when these plants are submerged the fronds float (96). This flotation of the fronds will materially modify the light factor, but quite apart from any such modification there is some evidence that in *Ascophyllum* the hydrostatic pressure of the bladders may be a factor modifying the lower limit of this species (51).

The relation between assimilation, respiration and littoral zonation is a problem that has only recently commenced to be explored (57, 82, 102, 121, 124, 159), but the higher the alga grows on the shore the shorter the time available for metabolism and growth, and when this factor can be translated into suitable terms it will probably prove to be a causal factor of first importance. It has already been shown that considerable photosynthetic gain is possible during periods of exposure (243), and that algae respond in three ways in respect of the effect of water loss on assimilation and growth (114). The results so far obtained for the relation of photosynthesis and respiration to changes of salinity (154) are somewhat conflicting. Thus a reduction in the salinity increases the rate of photosynthesis in *Fucus serratus* and *Ulva lactuca* (144), though the reverse has also been reported for *Ulva* (77). Reduction also increases the rate of respiration in *Fucus serratus* and

Laminaria digitata, whereas *Fucus vesiculosus, Enteromorpha* sp. and *Porphyra* sp. are not affected (100). The exact status to be allotted to the animals in their effects on the zonation has yet to be worked out in detail, although this aspect has not been wholly neglected (45, 116–118, 236, 237, 250, 255); at present there is still some divergence between botanist and zoologist (238). It is, however, very doubtful that many workers would subscribe to the view that the biotic factor is a master factor (194). As marine co-operative research becomes the rule rather than the exception this aspect should become clarified.

In summing up the position it seems that the evidence is more promising with regard to the determination of the upper limits of species, and at present it is very difficult to suggest factors that control the lower limits. It is doubtful that any weight can be attached to the suggestion (87, 122) that at lower levels increases of salt concentration are significant, though so far as green algae are concerned it seems that the concentration of sea water is the optimal one for their assimilation (78) . The lower the position on the shore the more favourable the environment becomes for the growth of seaweeds, and it may be competition between species for space and light that is the essential causal factor at lower levels. Here the comparative rates of growth and reproduction of the different species will be important, and also the effect of epiphytes, but our knowledge of these factors is hardly extensive. In general, variations in pH do not seem to be important, though in one species of *Fucus* successful growth of the sporeling takes place only over a relatively narrow range (81); this aspect demands further enquiry. The view that the upper limit of *Laminaria digitata* is correlated with the superficial position of sporangia and their exposure to water loss, and also that the lower limit of *Fucus serratus* is set by the thallus being unable to contract sufficiently to force gametes to the surface (105), can scarcely be entertained, since it is obvious that unless the sporelings can grow, neither of these two conditions can be involved. The power of species to resist desiccation decreases the lower they grow (6, 167), but this, in the case of the fucoids, appears to be bound up with the microchemistry and microstructure of the cell wall (93, 264): the lower the species on the shore the thinner are the cell walls and the less the amount of fat they contain.

So far, only the factors controlling littoral zonation have been considered. For the sublittoral there is general, though not universal, agreement that light penetration is the prime causal factor. This operates in its effects on assimilation rate, and this has also to be related to respiration (43, 57, 58, 82, 114, 121, 141, 160–164, 171, 221, 222). A critical depth for any species is obviously that where assimilation becomes equal to respiration (the compensation point). This depth for any species may be modified indirectly by the effect of such external factors as wind, which makes the water rough, and clouds (57, 222, 252). Below the compensation point depth, wear is not wholly replaceable, and it is not improbable that the depth varies with the age of the plant; at present we do not know how it may also vary from season to season and from locality to locality.

So far as actual light absorption is concerned it would appear that the colour of the alga acts in a physical rather than in a physiological manner (163, 221), and in this particular respect the distinction into sun and shade algae is of very considerable importance. The inter-relationships, however, may be distinctly complicated: thus red algae from well lit habitats (sun forms) in deep water possess no advantage over shade Chlorophyceae; similarly, Rhodophyceae and Phaeophyceae from the same horizon do not differ much in their capacity for photosynthesis. The recent discovery that *Laminaria cloustoni* exists in three physiological races, sun form, shade form and deep-water form, alters our concept of its behaviour around our coasts. The lower limit to which the species extends in any locality will depend on the physiological form present. This is a problem that no one has yet attempted to survey and solve.

The correlations between light penetration, absorption, assimilation, salinity and temperature are only just beginning to be worked out, and it is evident that the results (58, 102, 130, 172) may throw considerable light not only on the zonation to be observed in any one locality but also upon the geographical distribution of the species. There already appears to be some correlation between the temperature giving maximum assimilation and the average temperature during the month of maximum growth (58), whilst the great development of fucoids in the Arctic is probably due to the indirect effect of lowering the temperature, thus leading to an excess of assimilation over respiration (102). The fact that some algal growth can take place in temperate waters during winter and spring is due to the low respiration rate, so that in spite of the low light intensity assimilatory gain is possible (186). Latitude is also said to be significant because in the warm seas the compensation point is reached sooner with depth, but this result hardly tallies with the fact that the algal vegetation of warm water often descends to a greater depth than that of cold.

Whilst light may be the main causal factor in controlling the sub-littoral zonation, there are other presence and absence or modifying factors. Changes in the substrate will act as a presence or absence factor, whilst the existence of a strong swell, *e.g.,* the Pacific coast of North America, or of strong currents, *e.g.,* around the Orkney Islands, results in the extension of the large Phaeophyceae to greater depths. This factor must operate through increasing either the aeration, and hence respiration and assimilation (83), or the amount of nutrient salts available over a given period, but quantitative data are lacking. A change in the nutrient salt balance due to the mingling of river water is said (142) to be responsible for the distribution of *Laminaria ochraleuca* in the region west of the Cherbourg peninsula.

AUTECOLOGY AND RECOLONISATION

Autecology, *i.e.,* the ecological study of individual species, has in the time sequence followed upon synecology. Nearly all algal ecological studies have up to the present been synecological, and we are only now entering the phase of autecology. There is therefore an unlimited field available for future workers. Thus far work has progressed upon a few species, *e.g., Macrocystis pyrifera* (28, 265), *Saccorhiza bulbosa* (201), *Ascophyllum nodosum* (52), *Bangia fusco-purpurea* (257), *Porphyra* spp. (192) and *Chondrus crispus* (249). Even here, however, difficulties are apparent, and the study of one species in a single locality is not adequate: for example, *Ascophyllum* at Aberystwyth appears to produce one bladder and its associated thallus per season, whereas in other places (154) it may produce more.

A group of algologists in Great Britain are at present experimenting with the production of "ecological passports" for individual species; it is hoped that these may form the basis for a biological flora which would in essence be autecological in character. An earlier attempt (157) along similar lines was too premature to be successful.

Studies on the recolonisation of artificially denuded areas (19, 52, 117, 176, 192, 261) have already shown that much valuable information can be gained, and the technique should undoubtedly be extended. It is extremely likely that such studies will throw some light upon the factors determining the characteristic zonations that can be observed. In European waters the sequence of recolonisation in the areas studied appears to be much the same, apart from one exception (117), though it may be influenced by the season of the year (192): diatoms generally return first, followed by Chlorophyceae, usually species of *Enteromorpha,* and then the Fuci, the oospores of which secure a hold in the green chlorophycean mat which is also said to provide the necessary humid environment. If the substrate is sandy the Fuci return sooner because sand provides a better foothold for the oospores than bare rock. It is interesting to note that *Ascophyllum* does not always reoccupy the same zone that it previously did but often returns at a slightly higher level (52). From studies of bared areas evidence is also forthcoming of competition between *Balanus balanoides* and *Fucus vesiculosus,* often in favour of the former.

In the Pacific the diatom stage is succeeded by a hydroid phase, then *Ectocarpus,* a pre-kelp phase, and finally the kelps return (261). In warmer waters where brown rockweeds are not present the sequence is naturally somewhat different (19). In South Africa the first two stages are the same as in Europe but the third is dominated by *Porphyra capensis.* On the warm east coast this is followed by *Ulva lactuca,* whilst on the colder west coast the next colonist is *Iridaea capensis* followed by *Splachnidium rugosum.*

How far the succession can be modified by environment has yet to be fully worked out: in at least one area (192) it is influenced by the nature of the substrate (*e.g.,* whether wood, iron or concrete), tide-level (*e.g., Ulothrix, Urospora* are the pioneers above mean sea level between March and May, and *Ulva, Porphyra* and *Enteromorpha* below), aspect, angle of slope (*Enteromorpha* occurs on vertical and *Porphyra* on horizontal iron surfaces) and competition between plants and animals (*e.g.,* algae do not appear to grow on the tubes of *Pomatoceros*). There is no doubt that further work along these lines in carefully selected areas, *e.g.,* north and south of Cape Cod or Point Conception, or east and west of the Cape of Good Hope, would produce extremely valuable results.

GEOGRAPHICAL DISTRIBUTION

A vast field of research is still available in this direction. Publication of floras from different parts of the world proceeds, and geographical work can be founded upon them. Very little (94, 106, 108, 131, 132, 184) has been added since the pioneer work of the older algologists (22, 26, 27, 112, 113, 153, 155, 168, 226), and it is evident that here, as in nomenclature, there is the possibility of confusion arising unless future work is co-ordinated; in addition, the distribution of characteristic communities (184) has yet to be studied in detail. It is a *sine qua non* that local variations in a flora are quite insignificant as compared with the diversities that are shown by different regions. Thus the algal flora of the east and western north Atlantic and of the North Sea forms a single entity; the vegetations of the West Indies and of the Mediterranean represent two other units; the flora of the Pacific can be divided into north, central, south and so on. The warm tropical waters of the central Atlantic and Pacific form an impenetrable belt for many algae, and hence there is a marked diversity between the cold water floras of the two hemispheres.

Attempts have been made to divide the algal floras of various regions into their component elements (216, 217), but later workers (22, 27, 66, 112, 179) have not always followed the original schemes, and this is leading to confusion:

Smith (235) Setchell (216, 217) Hoyt (101)	Borgesen (22, 27) Jonsson (112)	Okamura (179)	Feldmann (66)
Boreal	Arctic		
	Sub-arctic	Subarctic	
Upper boreal	Boreal arctic	Japan & California	
	Cold boreal		
N. temperate	Warm boreal	Temperate	Boreal Atlantic
N. subtropical		Subtropical	Tropical Atlantic
		Tropical	Pan tropical
Tropical			
S. subtropical			
S. temperate			
Upper Austral			
Austral			
		Cosmopolitan	Cosmopolitan
		Indo-Pacific	Indo-Pacific
		Japan-Okhotsk	Mediterranean

It is very desirable that in the future the segregation of floras into their component elements should follow an agreed scheme. An arrangement somewhat like the following, based upon the schemes already proposed, will ultimately prove to be necessary, though doubtless additions and emendations will be forthcoming as our knowledge increases:

Proposed geographical elements

Arctic		Antarctic	
Subarctic		Subantarctic	
Boreal arctic		Austral antarctic	
Cold boreal Atlantic	Cold boreal Pacific	Cold austral Atlantic	Cold austral Pacific
Boreal Atlantic	Boreal Pacific	Austral Atlantic	Austral Pacific
North Subtropical Atlantic	North Subtropical Pacific	South Subtropical Atlantic	South Subtropical Pacific
Tropical Atlantic		Tropical Pacific	
Pantropical		Cosmopolitan	
Caribbean		Arabian	
Indo-Pacific			

The problem of endemism in marine algae has still to receive detailed attention. Lists of endemics have been published from various areas (20, 22, 26, 27, 65, 66, 115, 248), and these might well be studied in the light of a modified age and area hypothesis (260), bearing the limitations of the theory in mind.

Attention has been directed (245) to the existence of vicarious pairs of species and the light they can throw upon the arrangement of past land masses. One may suppose that during the glacial period in the northern hemisphere an arctic flora penetrated to France (77) and that some elements were left behind in the subsequent retreat, whilst the Baltic flora is regarded (145) as a remnant of an Atlantic flora established during the later *Littorina* period. There are many comparable problems that still require attention. Thus it is likely that the distribution of *Macrocystis* and of other algae may throw some light on the validity or otherwise of Wegener's hypothesis of continental drift. Another problem is offered in the fact that though most species of *Lessonia* are mainly circumantarctic, there is one, *L. laminarioides,* recorded from Japan and Siberia. How did this eventuate?

The important part played by temperature in controlling the distribution of algal species has been thoroughly established (210–214, 231), and several examples are now known where there are distinct differences associated with sea-water temperature changes north and south of certain points, *e.g.*, Cape Cod (215), Point Conception (217), Coast of Spain, South Japan sea (77) and South Adriatic (204). Recently another example has been carefully and completely illustrated from South Africa (236, 237). Here the fauna and flora on the east and west coasts differ markedly, the east having a pronounced warm component and the west an equally pronounced cold water component with some species ubiquitous and a few confined to the relatively short south coast. Not only is there a difference in the composition of the vegetation, but changes in the zonation can also be followed from east to west or *vice versa.* This investigation indeed forms a model of what is wanted in other suitable parts of the world. A rather less intensive study for certain species that occur on both sides of the English Channel has produced some promising results (72). *Cystoseira ericoides* and *Bifucaria tuberculata* reach their northern limit in southern England; there they tend to be restricted to gullies and are very sensitive to changes in environmental factors. On the French coast light is regarded as the dominant factor in controlling the distribution of *Bifucaria.* In some of the species studied it is evident that a wider area would need to be considered.

Certain species of *Laminaria* and *Macrocystis* can be used as indicators of cold water currents (106, 108), whilst it is also possible, if desired, to group the algae according to their responses to temperature changes, though it is doubtful that this treatment is really successful in the light of our present knowledge. A rather specialised, though extremely interesting, subject is the distribution of the free-living forms of algae. Sometimes it is not difficult to demonstrate what happens (203), but on other occasions a peculiar form, *e.g., Pelvetia canaliculata* forma *libera,* may occur in widely separated districts (Chapman, unpub.), and this opens up many interesting problems.

Among the plankton the oceanography of the Dinoflagellate genus *Ceratium* has recently been carefully studied (86), and it has transpired that there is no correlation with salinity or temperature. There is some evidence, at present not wholly adequate, that concentration of organic metabolic products determines distribution, whilst number of species and density are obviously related to the relative phosphate content. This type of work, however, is still in its infancy, and there is much more to be done.

LIFE-FORM

A study of life-form in the phanerogams may be a useful tool in the hand of the ecologist so long as the limitations of the method are fully realised. If a suitable life-form system for the algae could be evolved it would prove extremely useful in providing a quantitative picture of the vegetation, in comparing the floras of two or more areas, and perhaps in reflecting the type of environment. Raunkaier's system was based on the means of surviving the unfavourable season, but such a problem is not common to the majority of marine algae, though some have a narrow temperature range (213) and survive unfavourable temperatures either as spores or dwarf plants or in a denuded state. The present position has been aptly summarised (66) as follows: "Les quelques tentatives qui ont été faites à ce propos n'ont pas eu le succès désirable, et les classifications proposées jusqu' ici n'ont guère été utilisée que par ceux qui les avaient imaginées".

The earliest attempts at classification were based, as one might suppose, upon morphological criteria. The most recent developments can be regarded as dating from 1905 (182), in which bush and tree forms, net forms, whip forms, leafy forms, *etc.*, were described. Very little further progress was made until 1926 when a new wave of classifications was initiated with a scheme (216) based upon a study of coral reefs in the Pacific. This scheme also differed from the earlier one in that its basis was largely ecological: thus there were forms nestling into hollows (phloladophytes), forms growing under or in the shade of rocks (skiarophytes), surf-loving species (cumatophytes), boring algae (tranophytes) and so on. A year later another scheme was propounded (79), also based upon a study of warm-water vegetation (the Mediterranean). In this there was a reversion to the morphological basis, and it also possessed a further disadvantage in a somewhat loose terminology. Some of the groups also were based upon length of thallus, which may vary considerably for one and the same species in a single area, *e.g.*, *Laminaria cloustoni.*

The next classification to be proposed (88) was also based upon morphological criteria. It was, however, more ambitious because its author, realising the close inter-relationships on the shore between plants and animals, sought to make his scheme embrace both. So far as the algae were concerned the arrangement was very similar to that of 1905.

In 1931 it was suggested (123) that the criteria used for phanerogams, *e.g.*, method of perennation, could be employed. The scheme was not extended into any detail, and the four primary groups (perennials, pseudo-perennials, annuals, casual annuals) would require considerable subdivision. If method of perennation is to be adopted as the most favourable arrangement, then the classification suggested in 1937 (66) must be regarded as the most satisfactory so far produced. This is perhaps worth reproducing:

ANNUALS

a. Species found throughout the year: spores or oospores germinate immediately. Ephemerophyceae, *e.g.*, *Cladophora* sp.
b. Species found during part of the year only.
  i. Algae present during the rest of the year as a microscopic thallus. Eclipsiophyceae, *e.g.*, *Ectocarpus, Litosiphon.*
  ii. Algae passing the unfavourable season in a resting stage. Hypnophyceae, *e.g.*, *Nostoc, Coleochaete, Ulothrix, Porphyra, Dudresnaya.*

PERENNIALS

a. Found entire throughout the year
  i. Frond erect. Phanerophyceae, *e.g.*, *Fucus, Sargassum.*
  ii. Frond a crust. Chamaephyceae, *e.g.*, *Peyssonnelia.*
b. Only a portion of the frond persisting a whole year
  i. Part of the erect frond disappears. Hemiphanerophyceae, *e.g.*, *Himathalia.*
  ii. Basal portion of thallus persists. Hemicryptophyceae, *e.g.*, *Cladostephus, Corallina.*

The success of such a classification would depend on the importance that may be ascribed to perennation in an environment where conditions are generally extremely uniform. The ideas and terminology that have proved successful for the angiosperms may not be equally satisfactory for such a wholly different group as the algae. The present reviewer is inclined to the opinion that a classification based upon environment or mode of life is likely to prove more successful and more useful to ecologists. Furthermore, any system of life-form for algae should include the fresh-water and terrestrial species, if it is to be complete, and the majority of schemes so far proposed are lacking in this respect. A scheme propounded in 1939 (33) is admirable in so far as it applies to fresh water and terrestrial forms, and if these ideas could be incorporated in a general scheme the result should approach what is really required.

It is probable, therefore, that a scheme somewhat on the lines of that propounded by Setchell (216) and combined with that of Cedergren (33) would form a useful synthesis. At present there is no doubt that a satisfactory life-form scheme has yet to be devised. No serious attempt has been made to construct biological spectra for different regions, although the reviewer has attempted this (unpublished), using a life-form scheme that is a modification of Feldmann's. Certain features emerge from comparisons of different areas. Thus rocky sea shores are characterised by a high percentage of annuals and epiphytes, salt marshes by a high percentage of free-living forms and soil algae, bodies of fresh water by a high proportion of plankton (small forms) and pleuston (large floating forms), and warm marine regions by numerous calcareous forms. Apart from its value in comparing the floras of different regions there is good reason to believe that a successful scheme would also be very useful in comparing the vegetation belts at different levels on the sea shore.

NOMENCLATURE

The present usage of nomenclature in marine algal ecology is rapidly leading towards chaos, and it is most important that some authoritative decisions be reached in the near future, as an earlier attempt (111) was too premature. At present investigators adopt their own nomenclature which is generally some variant of the terminology used in land ecology. There are two main problems here which need to be solved. There is first the delimitation of the regions of the shore, *e.g.*, littoral, supralittoral and infra- or sublittoral (119), and then there is the nomenclature to be applied to the communities and belts of vegetation. In the latter case we are required to decide whether the terms used in land ecological practice should be retained, or whether the conditions are such, *i.e.*, the close inter-dependence of plants and animals (255), that a completely new set of terms should be introduced. If the former procedure is adopted it is important that workers in this field agree about the nature of the vegetation with which they are dealing, *e.g.*, does the vegetation represent a climax or is it fundamentally seral in character? The individualistic concept (89) has not been generally adopted by ecologists, and there seems no reason to believe that it would specially apply on the seashore.

Delimitation of the regions of the shore has been brought into confusion by the frequent use of the vegetation for purposes of demarcation (208). This is unsatisfactory (88), even if the vegetation is correlated with the physical conditions. Here it is more important than ever that there should be agreement between botanist and zoologist. In the past the boundary between the *Fucus serratus* and the *Laminaria* or red algal zone has often been regarded as marking the lower limit of the littoral. Some zoologists, on the other hand, have placed this lower limit at 40 metres (3) or even deeper (29). The portion of the shore, the littoral, that is exposed daily is obviously an important region, and yet its boundaries are almost as hard to fix as those of a species. If it is based upon physical characters the upper limit is often regarded as being normal high water line (208), but this would seem to be far too ambiguous. It could, for example, refer to high water mark of ordinary tides (*e.g.,* mean average tides), or it could apply to high water mark of ordinary spring tides. Another modification (128) is associated with the raising of the upper boundary through wave action (spray), thus giving what is termed a physiological high water mark or "litus" line (228). The spray zone (45, 109) could equally well be placed in the littoral or in the supra-littoral (66, 187). It probably matters little which usage is adopted so long as we can achieve some degree of uniformity and reduce the personal element. At present it would seem that a littoral extending from H.W.M. to L.W.M. of average spring tides would satisfy most workers. There would seem to be no reason for introducing such terms as "wash zone" (always covered), "splash zone" (areas where waves break) and "spray zone" (45), even though these do emphasise the effect of the tides. The use of L.W.M. of neap tides (88) for the lower limit would seem to be incongruous, though this is adopted by some workers. Even the definitions given above are not wholly satisfactory, and ultimately the best method for defining the littoral, when data are available, will be as that area lying between an upper limit with more than x submergences per annum and a lower limit with more than y emergences. A careful study is required in order to determine the exact values of x and y. In areas, such as the Baltic, where there is no tide, the term "littoral" is commonly applied to the zone that is uncovered during the summer fall of water level or to 0.5 metre below M.L.W. (227). This usage will only lead to confusion, and a special term, such as "seasonal exposure zone", is obviously required.

Whatever definition of the littoral be finally adopted it is likely that there will be a zone between the lower limit and extreme L.W.M. of spring tides where there will be occasional exposure. It will probably prove desirable to regard this as an independent zone, the lower littoral or the upper sub-littoral. At the other end of the vertical scale the supra-littoral will extend upwards from H.W.M. of average spring tides and will include the spray zone and the region covered by storm and equinoctial tides. The upper limit of the supra-littoral will therefore vary from place to place, depending on exposure and protection, and will be determined by extreme H.W.M. of spring tides or the upper limit of the spray zone. The lower limit of the halophilous phanerogams has been suggested (66) as the demarcation line, but this would not operate in a salt marsh or mangrove area.

Below the littoral is the sublittoral. In certain areas this has been subdivided (4, 66, 147, 187), but a gradation is usually discernible, and this practice ought to be abandoned. Some workers have placed the lower limit of this zone at the depth where the algal covering ceases to be continuous, whilst others (21, 199) have adopted the lowest depth to which attached algae can penetrate. Another suggestion for the lower limit has been the line where sedentary animals commence to dominate the algae (88). In the tropics animals tend to be more abundant than algae from the lower littoral downwards, and so the lower boundary of coral reefs has been suggested as a useful means of marking the sublittoral (88). The confusion that can be caused by adoption of botanical or zoological subdivisions is immense (228), and it is evident that the lower limit of the sublittoral requires to be placed on a purely physical basis, *e.g.*, the percentage of light penetration. The limits cannot therefore be regarded as fixed conclusively, and further work on physical constants in relation to flora and fauna is necessary.

The sublittoral is succeeded by the elittoral which is regarded as extending down to the lower limit of plant life (66, 208), but this definition is complicated because some authors make a distinction between microscopic and macroscopic plant life (88). In the tropics this criterion has not been used, and in its place the lower limit of *Lithothamnia,* between 38 and 100 fathoms, has been proposed in its place. This is obviously related (69, 76) to a photic factor, and again it is desirable that this limit be based on a purely physical rather than on a biological basis.

Below the elittoral a continental or archibenthal region has been recognised, although it has been suggested that there is an intermediate profundal or sub-elittoral region characterised by the presence of dead vegetable nutriment. Finally the lowest region is the abyssal. The vertical extent of all these regions will vary from place to place, and at present data are greatly required.

Turning now to the other problem of nomenclature, a full appreciation of the position can be achieved only after a brief survey of past developments. The first attempt at marine sociology can be said to date back to 1812 (256), but in 1832 an important contribution (5) was made in which the close interdependence of plants and animals was recognised. This work had a considerable influence upon contemporary thought and was followed by many workers, especially on the continent of Europe, for over half a century. Somewhat later a monumental work (147) on the Adriatic fauna and flora appeared, dealing with both in considerable detail, whilst similar studies (75) appeared for Great Britain and France (250). Ten years later the first use of the term "biocenose" appeared in the literature when it was coined to describe the biotic community which is such a feature of the sea-shore. From 1860 to 1890 numerous works appeared by Scandinavian authors (90, 95, 126, 193, 199, 224, 225) who described many algal formations and associations. Most of these were modelled on an early work (119), and this unfortunately introduced the confusion in terminology that has never since been wholly rectified. The formation was defined (119) as "a small portion of the whole algal vegetation and characterised by the type of vegetation; generally they obtain their characteristic stamp through one or more of the predominant algae". This represents a grave departure from current ecological practice, and what the Scandinavians often termed a "formation" would today be regarded as an "association" or "consociation". This was, in fact, emphasised by a later writer (259) who, at the same time, propounded a scheme that was far too generalised. Some of the later Scandinavian workers (20, 21, 112, 128, 207) modified these earlier ideas, and one finds descriptions of Fucaceae and Chlorophyceae formations each containing one or more associations. It is important to notice, however, that with but few exceptions all the associations were characterised by the presence of a single dominant. The term "association" cannot correctly (140) be employed for a complex group such as the Fucaceae association (45) because the environmental conditions between the upper and lower species vary so widely. Whether the *Laminaria* association should likewise be discarded is very much more questionable because the species intermingle much more. Another anomalous treatment was used in describing the associations of San Juan (166) where a single name was given to a community containing more than one dominant. Formations were also sometimes regarded as composed of associations united together under the same or nearly the same ecological conditions; such aggregations are better described as belts.

After 1900 the majority of algal ecological workers adopted the terminology used by the Scandinavians, or else they employed (1, 84, 85) the non-committal term of "community". This term is preferable to the incorrect usage of other ecological terms. Introduction of more terms was probably inevitable, and so we find "modes" based upon degrees of salinity and degrees of exposure and "facies" based upon variations of substrate (8–10). Later the word "facies" (9) became used for portions of associations dominated by secondary species or modified by exposure (158, 181, 187). It was then (88) pointed out that the word "facies" was originally introduced by geologists to indicate differences in ground and therefore should not be used in any other sense. In recent works (65, 66) it has been employed in its proper geological sense, and it has been replaced by the term "fasciation" (223) to describe modifications of the association.

Terms have also been employed incorrectly. Thus "zone" has been employed for "association" and "association" for "fasciation". Additional confusion in terminology has often resulted from a too detailed analysis of a small region, with the result that far too many communities have been described with the status of an association. In one case no less than 57 algal associations were described from Woods Hole (53).

The concept of the biotic community has received rather more attention since 1900. Quite early biocenoses were discussed by zoologists in relation to differences of environment, and some biocenoses were also described from northern Germany (175) and Sweden (197, 198). Before considering this phase of development in more detail mention must be made of a very important paper (49) which has exerted a profound influence upon algal ecology. The most significant departure from previous practice was the basing of formations upon differences of substrate, *e.g.*, rock, sand, mud, salt marsh, whilst exposure and protection were also involved. A number of subsequent authors followed this example of nomenclature (91, 116, 117), but it is important to remember that at least two leading ecologists (112, 246) have specifically stated that the term "formation" should not be used in any other sense than that in which it is used for land vegetation, and that, however tempting it may be to name the association after the habitat, nevertheless the name should be based upon the dominant plant or animal species.

About 1924 two very important contributions (88, 180) appeared in which the biocenose concept played a prominent part. In New Zealand eight formations were described, each composed of a number of subformations or associations, though many of the latter were characterised by only one dominant. There was a tendency also to restrict the animals and plants to separate formations, whereas animals and plants can be co-dominant and coextensive, *e.g.*, a *Novastoa*-encrusting coralline association (50). In the second paper on Gullmar Fiord an association was defined as "a combination of species where one or more forms predominate so that the community gets a biotically determined "physiognomy". Usually, however, the associations there described possessed only one dominant. The term "variation" was introduced to describe modifications within an association, but in view of its accepted usage in genetics this term must be discarded in favour of "fasciation" or some other term. An horizontally extended association was called a "belt" and a vertical group of associations were collectively known as an "association complex". It was pointed out that the biocenoses were often two-layered, so that one had the equivalent of synusiae. Epiphytes formed a distinct problem, and though it was admitted that they might characterise an association, nevertheless they were commonly classed as variations (= fasciations).

These ideas all formed an important contribution to marine ecology, and they have been employed in at least one subsequent paper (50).

Since then a number of workers (39, 45, 109, 238) have insisted that the extensive belts on the shore are primarily based upon tidal phenomena, and one should not therefore use plant names for them, as the species may vary from place to place. At present our information is not sufficient to enable us to say exactly how the belts could best be delineated and named. The reviewer believes that ultimately they will have to be based upon either degree of exposure or upon critical levels (45, 52). One definition of the term "association" (192) is indicative of these ideas, though at present it is scarcely practicable. The association is defined as composed of dominants accompanied by other species whose presence is determined by responses to factors similar to those influencing the dominants. There is no doubt that this is one aspect of the subject in which considerable advances may be expected.

From 1930 onwards ecological accounts describing associations, usually with one dominant, from a number of areas have appeared (34, 52, 56, 66, 84, 85, 91, 140, 145, 192, 198, 218–220, 264). The influence of earlier work (49) is still evident (34, 145, 192) in a number of the contributions, but in some of them new ideas have been introduced. Thus it has been realised that if the vegetation of a rocky shore is regarded as a climax vegetation then one should employ the ecological terms "consociation", "society", *etc.* (34, 52, 195–198), though even these have not always been used correctly. The word "society" has been employed not only for local dominance of a subsidiary species but also to mark seasonal dominance (192). It is desirable that these two phenomena be regarded as distinct and the term "aspect" be applied to cases of seasonal dominance.

Some workers apparently do not recognise the existence of formations (1, 65, 66, 84, 85), whilst, by way of contrast, a very recent paper (232) describes in considerable detail the formations to be found in South America and the Subantarctic. These formations, which are based upon life-form or locality, are as follows:

1. Upper littoral formation of drought-resistant algae.
2. Lower littoral formation of surf-resistant Rhodophyceae.
3. Lower littoral formation on sheltered coasts.
4. Surf formation of larger Phaeophyceae.
5. Formation of large submarine Phaeophyceae.

6. Formation of giant Phaeophyceae with buoy-carried fronds.
7. Formation of deep-water Rhodophyceae.
8. Formation of crustaceous corallines.

If normal ecological practice were followed it is clear that many of the above are too small to warrant formation status.

The two most important recent contributions are both the work of zoologists (223, 236, 237) who, in general, consider that animals are of prime significance and that the algae merely mark fasciations of the animal communities. This view is probably too extreme, though it is being adopted by others (2). One group of workers (223) recognised four principal biomes, each containing a number of associations, whilst fasciation was employed for modifications of associations resulting from loss of or addition to the important species. Stephenson (236, 237) has a much wider approach to the whole subject because he rightly demands that a system be evolved that is, so far as possible, applicable to the whole inter-tidal region of the world, and he has made a first attempt with the inter-tidal regions of South Africa and Scotland. The same worker also advocates use of tides as a means of delimiting the zones, but he points out that until more tidal data are available we shall perforce have to employ biological units.

There is much less comparable work on the algal ecology of salt marshes, either because it is not so attractive or because it is more difficult. Earlier workers (49, 110) adopted the terminology used for rocky shores, whilst later workers (192) have either followed this usage or used the non-committal term of community (32). It was not until recently (36, 37) that the dynamic nature of the algal vegetation on salt marshes was emphasized and seral terminology introduced.

From the above all-too-brief summary it is clear that in the past there have been three views, from

a) those who base their terminology upon habitat
b) those who stress large units (mainly zoologists)
c) those who stress small units (mainly algologists)

There is, therefore, the problem of whether terminology should be based upon habitat or whether existing ecological terminology, as applied to land vegetation, should be employed more strictly than it obviously has been in the past. If the former view is adopted then a new terminology would need to be evolved[5] in order to avoid confusion, but there seems little or no reason why existing ecological practice should not be maintained. If this second viewpoint is accepted then any difficulty of having to decide between the alternatives *b* and *c* above will automatically disappear. The reviewer strongly recommends maintenance of normal ecological practice using the definitions given in a standard text (247). Associations will then be characterised by more than one dominant, consociations by one dominant, societies by a locally dominant secondary species, and so on. The term "aspect" should be used for seasonal communities, and the term "fasciation" need no longer be employed. The word "zone" should be left to the phytogeographers and "belt" employed for the horizontal bands along the shore; "storey" could also then be abandoned. Ultimately our aim should be to characterise the belts by physical features, *e.g.*, degree of exposure, expressed either as a percentage or in terms of maximum periods of non-tidal exposure (39).

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[5] New concepts for existing terms (140) would be unsatisfactory.

The vegetation of the rocky shore is probably best regarded as representing climax vegetation, although it could be regarded as a number of secondary successions because the life of the components of the vegetation is short-lived. This, however, is purely relative, for an oakwood could be regarded in the same light, though the length of time before complete regeneration occurs is longer. Climax terminology should therefore be used on rocky shores, and the following outline is suggested as a possible scheme upon which marine ecologists, both botanical and zoological, might base discussions (purely animal formations are neglected):

A. Large brown rockweed formation type.
  1. North Atlantic large brown rockweed formation.
    a) Exposed coast complex
    b) Sheltered coast complex
      Balanus—Pelvetia canaliculata association
      Littorina—Fucus spiralis association
      Ascophyllum—F. vesiculosus association
      Balanus—F. serratus—Chondrus association

The associations will each contain one or more consociations and societies together with some seasonal aspects.

  2. North Pacific large brown rockweed formation.
  3. South Pacific large brown rockweed formation.
  (Both these would be subdivided as in 1.)

B. Large submerged Phaeophyceae Formation type.
  1. North Atlantic *Laminaria* formation.
  2. North Pacific kelp formation.
  3. South Pacific kelp formation.
  4. South Atlantic kelp formation.
  5. *Sargassum* formation.

C. Coral Formation type.
  1. West Indies Coral formation.
  2. Indo-Malayan Coral formation.
  3. Central Pacific Coral formation.
  4. Australian Coral formation.

Both B and C would be subdivided into associations dominated by plants or animals or by both.

We know very little about the status of the algal communities on sandy shores and in estuaries, and until more work is available one cannot say whether they should be regarded as climax or seral vegetation. The algal vegetation of salt marshes and mangrove swamps is clearly seral in character, and nomenclatural practice should follow the usual rules. Thus for salt marsh algal vegetation one can postulate the existence of a North Atlantic sere, Baltic sere, Northwest Pacific sere, South Pacific sere, Western Mangrove sere, Indo-Malayan mangrove sere and perhaps others. These seres will be composed of a number of associes, consocies and socies, and in describing these it may be necessary to consider the algae together with the phanerogams; but as animals are commonly not predominant they will rarely enter into the naming of the large units.

CONCLUSIONS

One can regard marine algal ecology as being at a parting of the ways. Much will depend on the type and nature of the work in the immediate future. Some degree of co-ordination is a necessity, and in this subject more than anywhere else there is need of teamwork between animal and plant ecologists and the systematist. The close inter-relations between plants and animals on the sea shore demand that future work be teamwork; only thus will a final and satisfactory solution of nomenclatural problems be achieved. Ecologists have been criticised (183) for this lack of co-ordination, and marine ecologists are perhaps most guilty. Synecological studies will finally be understood only if they are succeeded by adequate autecological studies, and the time is now ripe for a definite attack on the individual species. Generally speaking, synecology has, up to the present, been concerned with relatively small areas, and future workers should aim at co-ordinating their results with those of their predecessors and co-workers. It is evident that many gaps in our knowledge remain, some of them fundamental in character, and these must be filled. In particular we require much more information about the tidal phenomena on all types of coast; we need information about the types of algal communities that exist in muddy bays, sandy shores and mangrove swamps. Insufficient attention in the past has also been paid to the smaller algae, especially members of the Cryptophyceae, Chrysophyceae and Myxophyceae, and there is no doubt that much remains to be discovered of the part played by these algae in the tropics, although some data are already available (18, 60).

Further study of physiological races in relation to distribution and environmental factors will be of considerable importance, whilst the development of a workable life-form scheme may indicate additional avenues for profitable research.

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